University of Alberta

Metabolic and genetic factors affecting feed efficiency (residual feed intake) in growing beef steers

by

Karin Schmid

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Abstract

Feed accounts for 60-70% of the total production cost in a beef operation; thus, improving feed efficiency will have a favourable impact on profitability and global competitiveness. This study examined metabolic and genetic factors underlying variation between individuals in feed efficiency measured via residual feed intake. Indirect calorimetry measurements did not concur with previous research in terms of methane and heat production. Urinary 3-methylhistidine concentrations (an indicator of myofibrillar protein breakdown) demonstrated no significant differences between efficiency groups. Microarray and quantitative real time polymerase chain reaction analyses were performed on rumen tissue samples to identify differentially expressed genes between efficiency groups. SH3 domain-binding glutamic acid-rich protein like 3 and apolipoprotein A-1 were identified as two potential candidate genes affecting feed efficiency. The results from this study provide a basis for further research on the genetic mechanisms contributing to differences in feed efficiency in growing beef steers.

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List of Abbreviations

ΔCT – Difference Between Number of Amplification Cycles of Internal Standard and Number of Amplification Cycles of Target

3-MeH – 3-Methylhistidine

ADG – Average Daily Gain

ADP – Adenosine Diphosphate

AP-1 – Activator Protein-1

APL – Acute Promyelocytic Leukemia

APOA1 – Apolipoprotein A-1

ATP – Adenosine Triphosphate

ATRA – All-Trans Retinoic Acid Induced Pathway

BTA – *Bos taurus* Autosome

BW – Body Weight

CT – Amplification Cycle

CXXC – Conserved Consensus Sequence

DE – Digestible Energy

DMI – Dry Matter Intake

EBV – Estimated Breeding Value

FCR – Feed Conversion Ratio

GE – Gross Energy

GIT – Gastrointestinal Tract

GRX-1 – Glutaredoxin-1

HDL – High Density Lipoprotein

HPLC – High Performance Liquid Chromatography

IGF-1 – Insulin-like Growth Factor-1

LCAT – Lecithin:Cholesterol Acyltransferase

ME – Metabolizable Energy

Met 3-MeH – Metabolic 3-Methylhistidine Concentration ($nmol/kg^{0.75}$)

NADPH - Nicotinamide Adenine Dinucleotide Phosphate, reduced form

NE – Net Energy

NE_m – Net Energy for Maintenance

NE_p – Net Energy for Production

NF-κB – Nuclear Factor Kappa B

QRT-PCR – Quantitative Real Time Polymerase Chain Reaction

QTL – Quantitative Trait Loci

RFI – Residual Feed Intake

SH3BGRL3 – SH3 Domain-Binding Glutamic-Acid Rich Protein Like 3

SNP – Single Nucleotide Polymorphism

TIP-B1 – Tumour Necrosis Factor Alpha Inhibitory Protein

TNFα – Tumour Necrosis Factor Alpha

Tot 3-MeH – Total 3-Methylhistidine Concentration (nmol/L)

Chapter 1

General Introduction

1.1. Introduction

Historically, the improvement of beef cattle by means of selection has focused upon maximizing profitable outputs such as growth rate, weaning weight, yearling weight, carcass weight, etc. (Crews et al. 2005). The concentration of selection pressure to produce cattle that are heavier at sale time has resulted in animals that tend to consume more feed to generate greater weight gains. While increased sale weights have led to greater producer profits, the costs incurred by increased feed consumption due to larger animals offset the profitability of both feeder cattle and the mature breeding herd.

In beef production, feed costs account for 60-70% of the total costs of production (Lindsay 2006). Of these costs, 50% of the total energy consumed in beef production is attributed to maintaining the mature cow herd (Montano-Bermudez et al. 1990). It is clear that reducing the input cost of feed would increase profitability, if it were possible for calves to maintain a similar growth rate while keeping the body size of the mature cows moderate. Improving feed efficiency (the ability of an animal to convert intake feed energy into energy for growth and production) represents the most viable method to decrease input costs (Herd et al. 2004).

It has been demonstrated (Archer et al. 1999; Arthur et al. 2001; Arthur et al. 2004; van der Westhuizen et al. 2004) that variation exists between individual animals in terms of feed efficiency. Traditionally, the most common method of calculating feed efficiency in cattle has been feed conversion ratio (FCR), which is defined as the amount of feed required to produce one unit of weight gain (Archer et al. 1999). Selection based

upon FCR tends to increase the overall size of mature cattle, as FCR is moderately heritable (h^2=0.29; Arthur et al. 2001) and there is a negative correlation between FCR and mature weight (Carstens et al. 2002). Since larger animals require more energy for maintenance (Jenkins et al. 1991), feed intake escalates to compensate for the larger body size.

More recently, a different measure of feed efficiency has garnered attention as a potential method to improve feed efficiency without sacrificing growth rate or increasing mature size. Residual feed intake (RFI) refers to the difference between an animal's actual feed intake and its expected feed intake based upon growth rate and body size (Archer et al. 1999). Defined in this manner, cattle that are more feed efficient will have a negative RFI value, while those that are less feed efficient will have a positive RFI value. Many studies have reported moderate heritability estimates for RFI, ranging from h^2=0.14-0.44 (Archer et al. 1999; Pitchford 2004), indicating that selection for feed efficiency using the RFI measurement is feasible. In addition, it has been demonstrated that RFI is phenotypically and genetically independent of mature weight (Herd and Bishop 2000). The obvious inference is that selection for improved feed efficiency using RFI will result in cattle that have a similar growth rate and mature size, while exhibiting a decreased feed intake compared to their unselected counterparts.

Essential physiological processes such as energy use and partitioning, feed intake, heat increment, protein turnover, feeding behaviour and activity, and overall tissue metabolism play a considerable role in the growth and development of beef cattle. These processes are all involved in the routine maintenance of the animal, and command energy usage priorities (Richardson and Herd 2004). After meeting maintenance requirements,

the residual portion of feed energy is utilized for production. The efficacy of a number of biological processes could contribute to a greater amount feed energy available for productive purposes (such as growth). The variation in RFI arising from processes such as protein turnover/tissue metabolism/stress, feeding patterns, body composition, activity, digestion, heat increment, and "other" uncharacterized factors accounts for 37%, 2%, 5%, 10%, 10%, 9%, and 27%, respectively (Richardson and Herd 2004). It is reasonable that most of the divergence between animals in RFI arises from fundamental differences in the manner in which energy is partitioned within the animal, as well as how much feed energy is available for maintenance versus productive purposes.

Energy is utilized by the animal to produce feces, urine, gases (predominately methane), heat, as well as to maintain standard essential body functions and activity (Baldwin and Sainz 1995). The amount of energy that these processes consume dictates the availability of energy for production. Reductions in both heat and methane production have been reported in low RFI steers (Hotovy et al. 1991; Herd et al. 2002; Basarab et al. 2003; Okine et al. 2003; Hegarty et al. 2004; Nkrumah et al. 2006), demonstrating the effect that energy conservation has upon feed efficiency.

In the same vein, it has been suggested that 67% of maintenance energy requirements are attributed to ion transport and the sustainment and turnover of protein depots within the body (Herd et al. 2004). Skeletal muscle (protein) in cattle comprises about 33% of the total body mass (Gopinath and Kitts 1984), and the efficacy of protein synthesis, degradation, and deposition may contribute to variation between animals in maintenance energy requirements. Similarly, the gastrointestinal tract (GIT) of ruminants is responsible for approximately 32-45% of total protein synthesis (Lobley et al. 1980)

and requires energy for digestion and nutrient transport as well; therefore, fluctuations in these processes between individuals would impact energy utility, and ultimately feed efficiency. Variation in the proportion of activity of the different modes of digestion (ruminal versus intestinal), as well as differences in the ruminal microbial population may also contribute to inequalities between animals in energy usage and feed efficiency (McAllister et al. 1994; Channon and Rowe 2004).

While the genetic mechanisms underlying energy partitioning and feed efficiency have been investigated (to varying degrees), a great deal of uncertainty remains concerning the role that genotype plays on metabolic processes. Candidate genes such as uncoupling protein-1, uncoupling protein-2, uncoupling protein-3, neuropeptide Y, and leptin have been linked with energy partitioning (Murdoch 2004). Similarly, leptin has also been associated with RFI (Nkrumah 2006). The discovery, confirmation, and manipulation of such candidate genes have become much more practical with the advent of high-throughput genomic technology, such as microarrays. Microarray technology is attractive to researchers as it allows for the opportunity to analyze thousands of genetic elements at once (Lehnert et al. 2006). This is especially useful when there is limited knowledge available to form a reasonable hypothesis as to the genetic mechanisms involved in a particular process. With a complete panel of genes to compare to the target sample, it is viable to examine the genetic differences between two treatments (e.g. feed efficiency), even without prior knowledge of the potential genetic factors involved.

Measures of feed efficiency, especially RFI, are of increasing importance to all aspects of the beef cattle industry. The greatest challenges lie in identifying the main metabolic and genetic factors that contribute to the variation between animals for this

trait, and in devising a cost-effective method for differentiating between efficient and non-efficient animals in terms of feed efficiency.

1.2. Research hypotheses

The present study tested the following research hypotheses:

1.2.1. Residual feed intake (RFI) is correlated with methane production, heat production, and energy partitioning of growing beef steers.

1.2.2. The energy required for the process of protein degradation is partially responsible for differences between animals in energy partitioning and feed efficiency of growing beef steers.

1.2.3. Upregulation or downregulation of currently uncharacterized genes are indicators of the individual animal variation in metabolic processes that contribute to the differences in RFI and feed efficiency of growing beef steers.

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Chapter 2

Literature review

2.1. Introduction

Improving feed efficiency in cattle may represent the most viable way to increase profitability, as feed costs account for 60-70% of the total production cost in a beef operation (Lindsay 2006). Consequently, these costs limit producer profitability. It is necessary to find ways to reduce feed costs without sacrificing animal nutritional status, growth rate, and final market weight. Feed efficiency is the proportion of total feed energy consumed by the animal and utilized for productive purposes (e.g. growth and milk production) after the animal's maintenance requirements are fulfilled (Herd et al. 2004). Traditionally, feed efficiency has been expressed using feed conversion ratio (FCR). Feed conversion ratio is the amount of feed necessary to produce one unit of weight gain (Archer et al. 1999). This measure of feed efficiency does not take into account the feed requirements for animal maintenance and growth (Carstens et al. 2002). Previous efforts to improve feed efficiency have focused on decreasing FCR, so less feed is needed to produce a similar gain. However, FCR is moderately heritable (h^2=0.29), and negatively correlated with growth rate (Arthur et al. 2001); hence, direct selection tends to increase growth rate as well as mature size (Carstens et al. 2002). This relationship between FCR and growth rate makes it difficult to determine if the variation in feed efficiency between animals is actually due to differences in metabolic processes, or simply discrepancies in growth rate and maturity pattern (Carstens et al. 2002). Therefore, the underlying causes of an improvement in feed efficiency are difficult to quantify using FCR. As well, the corresponding increase in the mature size of cattle

resulting after generations of selection against high FCR is detrimental to the reduction of feed costs, as larger animals require more feed for maintenance (Jenkins et al. 1991).

2.2. Residual feed intake (RFI)

More recently, research efforts have focused on a new measurement of feed efficiency, termed residual feed intake (RFI). Residual feed intake is defined as the difference between an animal's actual feed intake and its expected feed intake for its growth rate and metabolic body weight (Archer et al. 1999). Using this definition, cattle with a negative RFI value are classified as more efficient than those individuals with a positive RFI value. Reported heritability values for RFI vary widely (Table 2.1), but are generally accepted to range from approximately h^2=0.14-0.44 (Archer et al. 1999; Pitchford 2004).

Furthermore, RFI has been shown to be phenotypically and genetically independent of mature size and growth rate (Herd and Bishop 2000); therefore, the potential exists for feed efficiency to be improved through direct selection against high RFI without sacrificing growth rate or increasing mature cow size. The lack of correlation between RFI and mature animal size is promising, as RFI could be improved without incurring the increased feed costs associated with larger mature cows.

To illustrate the economic value of improving feed efficiency, Fox et al. (2001) reported a 43% increase in profits based upon a 10% improvement in animal feed efficiency, in contrast to an 18% increase in profits based upon a 10% increase in animal rate of gain. This difference in profit is mainly due to a significant decrease in the number of days on feed to reach the target weight gain of 600 lbs (Fox et al. 2001).

Similarly, Basarab et al. (2003) reported a difference in feed intake of 3.77 kg/day (as-fed) between the most efficient and least efficient steers (ranked using RFI). This 3.77 kg/day difference in feed intake resulted in a feed cost savings of $0.38/day (feed costs=$0.101/kg as-fed). Projecting these values onto Alberta's 2.4 million head of feeder cattle in 2003 indicates a savings of approximately $109 million in feed costs.

Archer et al. (2004) developed a model beef breeding system to evaluate the economic benefits of measuring RFI and feed efficiency. Gains in feed efficiency ranged from 8-36% over the base scenario after selecting breeding bulls based upon desirable RFI values. They established that profit would be maximized if the top 10-20% of bulls were measured for RFI. Overall profit was improved from the base scenario (no RFI measurement) by 9-33%, or $150-$450 per head.

These studies emphasize the economic benefits that selection against high RFI can impart to the feeder cattle industry. If similar profits are attainable during the feeding of the mature cowherd, RFI is extremely appealing financially. At present, the process of ranking feed efficient animals based upon RFI is expensive and impractical for both producers and feedlot operators, and generally, the biological mechanisms underlying differences in RFI remain relatively unknown. However, Richardson and Herd (2004) have suggested several sources that may contribute to the variation in RFI, including energy use and partitioning, feed intake, heat increment, protein turnover, feeding behaviour and activity, and overall tissue metabolism.

2.3. Energy use and partitioning

To properly understand feed efficiency and RFI, it is necessary to gain a thorough understanding of energy partitioning in cattle. Energy is required for many essential metabolic processes such as basal metabolism, voluntary body activities, thermogenesis, protein and fat metabolism, ion transport, vital organ/nervous function, and digestion (Archer et al. 1999). The maintenance requirements for energy must be fulfilled before any production (e.g. growth, milk production) can occur.

As feed passes through an animal's digestive tract, the total nutrient value of the feed is diminished. Gross energy (GE) is the amount of total feed energy consumed by the animal. Energy is subsequently dispersed through the production of feces, urine, gases (predominately methane), and heat, resulting in a functional residual portion (net energy or NE). Net energy can then be divided into two portions, net energy required for maintenance (NE_m - basal metabolism, voluntary body activities, thermogenesis, protein and fat turnover, ion transport, and vital organ/nervous function, and digestion), and net energy required for production (NE_p) for processes such as growth, gestation, and milk production (Baldwin and Sainz 1995 - Figure 2.1).

The NE_m requirements must be satisfied before any energy can be utilized for productive purposes. Since approximately 60-75% of total feed energy is needed to fulfill maintenance requirements (Archer et al. 1999), it seems likely that variation in feed efficiency between animals arises from differences in maintenance energy requirements.

The various processes that comprise maintenance requirements (basal metabolism, voluntary body activities, thermogenesis, protein and fat turnover, ion

transport, vital organ/nervous function, and digestion) demand different amounts of energy. The estimated proportion of each processes' contribution to variation in residual feed intake is displayed in Figure 2.2.

Evidence for variation between animals in energy expenditures and maintenance energy requirements is well documented (Archer et al. 1999; Arthur et al. 2004; Herd et al. 2004). Hotovy et al. (1991) employed identical twins to negate the influence of genetic differences between test subjects in an analysis of heat production and metabolizable energy required for maintenance in beef cattle. The results suggest that there is a genetic component to the variation between animals in heat production and metabolizable energy required for maintenance due to the high heritability estimates of h^2=0.75 and h^2=0.52, respectively (Hotovy et al. 1991). The high heritabilities in the above study indicate that selection for reduced heat production/maintenance requirements in beef cattle is possible.

Cellular energy production occurs with the mitochondria producing the bulk of ATP, facilitating cellular processes (Kolath et al. 2006a,b). The production of ATP occurs mainly through oxidative phosphorylation of ADP into ATP via the Kreb's Cycle and the electron transport chain. The efficiency of mitochondrial function or the electron transport mechanism could contribute to increased or decreased maintenance energy requirements. Kolath et al. (2006a) hypothesized that a relationship exists between mitochondrial respiration and RFI. The researchers also examined the probability of an "electron leak" impairing the operation of the electron transport chain. They discovered a superior degree of coupling between respiration and oxidative phosphorylation in the mitochondria of low RFI steers, suggesting a greater efficiency of electron transfer. In

addition, the researchers discovered no significant difference in the amount of electron leak between high and low RFI steers when expressed as a function of mitochondrial respiration rate. It was concluded that while mitochondrial function itself has little to no impact upon RFI, the efficiency of electron movement through the electron transport chain is impaired in high RFI steers (Kolath et al. 2006a).

The discovery of a link between the electron transport chain and RFI in steers led to further examination of the mitochondrial electron transport chain (Kolath et al. 2006b). The study focused on uncoupling proteins 2 and 3, as well as single nucleotide polymorphisms (SNPs) in mitochondrial DNA. Uncoupling proteins 2 and 3 were hypothesized to play a role in the uncoupling of oxidative phosphorylation by transporting protons into the mitochondrial matrix (Kolath et al. 2006b). Increased expression of uncoupling protein 2 and 3 would reduce the amount of oxidative phosphorylation occurring in the mitochondria, raising the energy requirement to produce ATP. High and low RFI steers showed no expression differences in uncoupling protein 2 or 3, which indicates that the proteins of interest do not play the hypothesized uncoupling role. SNPs in mitochondrial DNA would cause a decline in the performance of the electron transport chain (Kolath et al. 2006b). However, all 19 mitochondrial transfer RNA genes examined contained no polymorphisms in either the high or low RFI group, suggesting that mitochondrial SNPs and RFI are unrelated (Kolath et al. 2006b).

Many reports have linked body composition and RFI (Basarab et al. 2003; Arthur et al. 2004; Richardson and Herd, 2004; Tedeschi et al. 2006). Animals gaining proportionally more fat versus protein are likely to have a greater energetic cost attributed to that gain, as fat contains more energy per kilogram deposited (Robinson and Oddy,

2004). Hence, growing animals with low RFI values tend to be leaner than their high RFI counterparts, with no known significant detrimental effects upon meat quality (McDonagh et al. 2001; Nkrumah et al. 2004; Baker et al 2006). In mature cattle, Dicostanzo et al. (1990) estimated 192.9 kcal/kg and 20.7 kcal/kg to maintain depots of protein and fat, respectively. The maintenance energy requirement of protein is much greater than that of fat, due to the high rate of turnover. This result illustrates that a mature cow with a larger protein mass will have a greater maintenance energy requirement than a cow with a smaller protein mass (assuming fat mass is equal), due to the greater demand of protein turnover (Dicostanzo et al. 1990).

2.4. Relationship between residual feed intake (RFI) and protein turnover in skeletal muscle

As there is an energy cost related to both the synthesis and degradation of protein in cattle, it is reasonable to assume that the variation between animals in either the amount of protein synthesized and degraded, or the efficiency of the synthesis and degradation processes could have impacts on RFI and overall feed efficiency.

It has been demonstrated that cattle divergently selected for high and low growth-rates over 17 years differ in both yearling and mature weight by 30% (Parnell et al. 1997). It is also reported (Oddy et al. 1998) that calves from the high growth rate line also had a faster rate of protein accretion than did their low growth rate counterparts. In addition, the cattle from the high growth rate line demonstrated lower rates of muscle protein degradation than did the cattle from the low growth rate line. Researchers (Oddy et al. 1998) determined variation between high and low growth rate lines in the pattern of

protein deposition as feed intake increased. Those individuals with a high growth rate tended to increase protein accretion through decreased rates of protein turnover, while those with a low growth rate increased protein accretion by increasing protein turnover, with the rate of protein synthesis slightly higher than the rate of degradation. From these observations, it was concluded that the steers in the low growth line had inherently higher levels of protein turnover in hind-limb muscle tissue. The levels of subcutaneous fat between high and low growth rate steers in this experiment were similar, and therefore did not contribute to the difference between lines in efficiency of growth.

However, the results of Oddy et al. (1998) conflict with those of Dicostanzo et al. (1991) who reported observations for protein accretion in mature cows classified as inefficient, average, and efficient through the difference between expected and observed live weight gain. Inefficient cows had a higher rate of protein accretion (Dicostanzo et al. 1991) than did their efficient counterparts. There are a number of ways to explain the discrepancy between the two studies. Firstly, the animals examined in Dicostanzo et al. (1991) were mature cows, versus the growing steers used in Oddy et al. (1998). Growing animals have an elevated rate of protein turnover compared with mature ones (Owens et al. 1995), therefore the higher rates of total protein synthesis and degradation could account for the difference in protein accretion rates between the two studies. Secondly, Oddy et al. (1998) reported that inefficient steers demonstrated an increased rate of protein degradation, which explains the slower rate of protein accretion in that study. Thirdly, it is possible that inefficient cows in the Dicostanzo study (1991) had an increased rate of protein synthesis, resulting in increased rates of protein accretion, although specific measurements of protein synthesis and degradation were not analyzed

in that experiment. In both cases, clear dissimilarities exist in protein accretion between efficient and inefficient animals, although the exact mechanisms resulting in the difference in protein accretion between the two studies is presently unclear.

The results of Oddy et al. (1998) are supported by the documented energetic efficiency of protein accretion. Owens et al. (1995) reported an average energetic efficiency of protein accretion of 47% in beef cattle, with the remaining 53% being released as heat. This can be compared to the average energetic efficiency of fat accretion at 76%. The low energetic efficiency of protein accretion means that a great deal of the energy for protein synthesis and degradation is lost as heat. Lobley (1990) reported that 20-25 kJ of heat is released for every gram of protein synthesized by the animal. Similarly, Dicostanzo et al. (1990) reported that 88.6% of the total energy requirement for maintenance was used to maintain protein mass within the body. Protein turnover and accretion are energetically expensive processes that contribute to maintenance energy requirements and energy losses (as heat) in cattle.

The correlation between RFI and reduced rates of protein turnover in cattle is further evidenced by Richardson and Herd (2004). Growing steers in this study were divergently selected for RFI, with the high RFI steers (inefficient) having higher total protein plasma content than low RFI (efficient) steers. A negative correlation was discovered between RFI and protein gain ($r=-0.50$, $p<0.001$), which demonstrates that more feed efficient steers may have either a lower rate of protein degradation, or a more effective protein accretion mechanism. They also showed a positive association (regression coefficient=1.20 ± 0.35) between sire estimated breeding value (EBV) for RFI and blood urea concentration (Richardson and Herd 2004).

Blood urea concentration is an indirect indicator of protein degradation, as urea is a product of protein breakdown (Richardson and Herd 2004). The positive relationship between sire EBV for RFI and blood urea concentration indicates that inefficient steers have a greater rate of protein degradation. Hence, whole-body protein turnover is genetically associated with RFI (Richardson and Herd 2004).

In cattle, the muscle tissue (protein) comprises approximately 33% of the animal's total body mass, or 55% of the total empty body protein in cattle (Gopinath and Kitts 1984). The muscle tissue in the body is in a constant state of flux, requiring a steady source of energy for its maintenance. Increased levels of energy (through increased feed intake or increased levels of metabolizable energy in the feed) will result in protein accretion (increased muscle mass), assuming that there is an available source of amino acids (Owens et al. 1995). The efficiency of protein accretion determines how much production energy is consumed by this process. If less energy is required for protein accretion, whether through decreased levels of degradation or decreased levels of both synthesis and degradation, the animal can consume less feed, while maintaining the same growth rate. As protein synthesis requires approximately five times more energy than protein degradation (Lobley et al. 2000), it seems a plausible assumption that that low RFI animals will show a decrease in both protein synthesis and degradation (to different degrees) or a decrease in protein degradation alone.

Muscle protein degradation in cattle can be measured non-invasively in vivo by analyzing the concentration of 3-methylhistidine (3-MeH) excreted in the urine. This method has been validated in cattle (Nishizawa et al. 1979; Harris and Milne 1981; McCarthy et al. 1983), illustrating that 3-MeH is quantitatively excreted in the urine and

not reutilized by the body for further protein metabolism. When myofibrils (the connective structure of muscle, primarily actin and myosin) of muscle tissue break down, 3-MeH is released; therefore, the measurement of 3-MeH gives an accurate estimate of myofibrillar protein breakdown (Harris and Milne 1981). Commonly, myofibrillar protein breakdown is expressed in a ratio of urinary 3-MeH content to urinary creatinine content to correct for differences in myofibrillar protein mass; however, this method is criticized because animals must be on similar nutrient intakes to garner accurate estimates (Gerrits et al. 1998).

There is a relationship between between overall animal size and excretion of urinary 3-MeH (Lobley 1998). Larger framed animals tended to excrete more 3-MeH than smaller framed cattle at similar rates of average daily gain (Lobley 1998). This is likely because larger animals generally have a larger muscle mass, and consequently more myofibrillar protein turnover, though varying maturity patterns rather than discrete differences in protein turnover may be the root cause.

As shown in Table 2.2, larger framed animals excrete significantly more 3-MeH, rendering it necessary to correct for differences in muscle protein mass, while keeping in mind the criticisms of the 3-MeH to creatinine ratio. It is of note that the animals in the above study were of the same age, and as the Charolais breed is later maturing, the differences in 3-MeH excretion may stem from differences in physiological maturity (Lobley 1998).

Other sources of endogenous protein may also contribute to 3-MeH content in urine (McCarthy et al. 1983). It has been shown that the gastrointestinal tract (GIT) and skin contributes 25% of total urinary 3-MeH excretion in rats (Nagasawa 1995). These

results are in conflict with the results of van den Hemel-Grooten et al. (1997), whose results stated that the portal drained viscera contributed less than 6% of total urinary excretion in pigs. These results are for monogastric animals, and cannot be directly applied to ruminant animals, for which less data exists. Currently, the extent to which the GIT and portal drained viscera (e.g. liver) protein sources contribute to total 3-MeH concentration in ruminants is relatively unknown, although it has been estimated that approximately 7% of the total urinary 3-MeH excreted in cattle could be a result of protein turnover in the GIT (Gerrits et al. 1998). Due to the apparently small contribution of protein turnover in tissues other than skeletal muscle to total 3-MeH excretion in ruminants, urinary 3-MeH concentration remains an accepted method of quantifying myofibrillar protein breakdown in the skeletal muscle of cattle (Harris and Milne 1981; McCarthy et al. 1983; Gopinath and Kitts 1984; Gerrits et al. 1998).

2.5. Relationship between residual feed intake (RFI) and gastrointestinal tract (GIT) metabolism

2.5.1. Protein turnover

In cattle, the gastrointestinal tract (GIT) accounts for 32-45% of total protein synthesis, while only representing 5% of total protein mass (Lobley et al. 1980), making GIT metabolism a probable candidate for sources of variation in RFI. However, the complexity of the ruminant digestive system causes difficulties in accurately measuring the rate of protein turnover in the GIT, and consequently there is a notable lack of published literature information on the subject. In addition, available research (Nishizawa et al. 1979, Lobley et al. 1980) deals with a very small sample size and

therefore the extrapolation of the results onto the entire cattle population may be unrealistic.

Lobley et al. (1980) compared whole body and tissue protein synthesis on two growing heifers and one mature dry cow using a constant infusion of radiolabelled amino acids. This method can be problematic in determining exact values of protein synthesis, as quantifying the magnitude of the specific radioactivity of the labeled amino acid is a long and difficult process (Lobley et al. 1980). Muscle tissue protein synthesis in the growing heifers contributed an average of 17% to whole body protein synthesis, while GIT tissue contributed an average of 37%. For the mature cow, contributions of muscle protein synthesis and GIT protein synthesis averaged 18% and 42% respectively (Lobley et al. 1980). These results indicate that protein synthesis in the GIT tract of cattle is more energetically expensive than protein synthesis in muscle tissue, and may have more of an impact upon overall feed efficiency. However, the above results regarding muscle protein synthesis are contradicted by the discussion in the previous sections of this review, which indicate that total protein turnover of muscle tissue may have more of an impact on energetic efficiency than indicated in the study of Lobley et al. (1980), which only examined protein synthesis.

McBride and Kelly (1990) reviewed the energetic contribution of protein synthesis and degradation in the GIT to total body energy expenditure based upon oxygen consumption. Approximately 4.0-4.6% of total body energy expenditure is due to protein synthesis in the GIT, while only 0.9% of total body energy expenditure is due to protein degradation in the GIT (McBride and Kelly (1990). Therefore, 4.9-5.5% of total body energy expenditure is attributed to protein turnover in the GIT. While the values of

Lobley et al. (1980) and McBride and Kelly (1990) cannot be directly compared to each other, they both indicate that protein metabolism in the GIT contributes to overall maintenance energy requirements. The extent of that contribution is unclear; however, variation between animals with regards to protein turnover in the GIT could impact RFI.

2.5.2 *Methane production*

Digestion in ruminants is responsible for approximately 10% of the total variation in RFI in beef cattle (Figure 2.2). As such, discrepancies in digestive efficiency and/or rumen metabolism between individuals could impact RFI. Due to the myriad of microorganisms inhabiting the rumen, it is difficult to assemble an accurate picture of ruminant digestion and how it relates to feed efficiency in cattle.

Microorganisms within the rumen are responsible for the majority of feed breakdown in cattle (McAllister et al. 1994). A byproduct of the digestion of feed by various microorganisms is methane (Immig 1996), a greenhouse gas. As much as 8.4% of gross feed energy can be lost to the atmosphere as methane (Harper et al. 1999; Figure 2.1). As such, a reduction of methane produced by the microorganisms in the rumen would result in animals with a greater amount of feed energy to use for maintenance requirements or productive purposes.

Many studies (Herd et al. 2002; Okine et al. 2003; Hegarty et al. 2004; Nkrumah et al. 2006) have linked low RFI animals with reduced methane production. This relationship between RFI and methane production is expected, since methane production is highly reliant on the amount of feed consumed by the animal (Blaxter and Clapperton 1965). Therefore, the low RFI animals that consume less feed should have a lower

methane production. Herd et al. (2002) observed a 15% reduction in enteric and fecal fermentation methane production in low RFI cattle. These results are similar, if slightly greater in magnitude, to those of Hegarty et al. (2004), who predicted that a 1.0 kg/d decrease in the estimated breeding value (EBV) for RFI would accompany a 7% decline in methane production. Nkrumah et al. (2006) discovered that low RFI steers experienced a 28% drop in methane production compared to high RFI steers.

Methane production is influenced by the type of volatile fatty acid produced in the rumen (Boadi et al. 2004). The production of acetate and butyrate leads to more hydrogen (H_2) available for methanogenesis, while the production of propionate is a H_2 sink (Boadi et al. 2004), competing for H_2 with methane. Feeding a high forage diet results in a slower passage rate of feed through the rumen, as well as higher concentrations of acetate and butyrate, instigating an increase in methane production. Nkrumah et al. (2006) observed that the feeding duration and feedbunk attendance of high and low RFI steers was significantly different, with low RFI steers exhibiting fewer and shorter visits to the feedbunk per day. This result is noteworthy as low meal frequencies increase propionate production, and amplify shifts in ruminal pH between feedings, both of which are detrimental to methanogen survival (Sutton et al. 1986). The decreased feeding frequency of low RFI animals will intrinsically result in reduced methane production, especially if fed a high concentrate diet.

Methane production has become a hot-button topic in recent years. With a shifting focus in the agriculture industry towards more sustainable and environmentally friendly practices, reducing greenhouse gas emissions is a priority. Approximately 7.3% of all greenhouse gas emissions in Canada originate from the agriculture industry (as of

2004), with 44% of that total being attributed to the enteric fermentation of livestock (Environment Canada 2004). A reduction in methane production via selection against high RFI would help reduce the contribution towards greenhouse gas emissions by the beef industry, resulting in a more environmentally sustainable production system.

2.5.3. Starch digestion

There have been reports (Channon and Rowe 2004; Channon et al. 2004) of variability in starch digestion between high and low efficiency cattle. Fermentation of starch in the rumen leads to the production of methane gas, versus digestion of starch in the small intestine, which results in very little methane gas and energy loss (Channon and Rowe 2004). Ruminal fermentation of non-structural carbohydrates (starch) can result in gross energy losses of 13-18% (Harmon and McLeod 2001), even though the digestion of starch results in increased propionate production. The advantage of small intestinal digestion of non-structural carbohydrates is the almost complete lack of methane production. It appears that more feed efficient animals may ferment more starch in the small intestine, rather than digesting it in the rumen, resulting in reduced energy losses, and improved feed efficiency.

2.5.4. Dry matter digestibility

Increased feed intake generally leads to decreased dry matter digestibility in cattle (Herd et al. 2004). However, there is variance in the abilities of high efficiency and low efficiency cattle to digest dry matter, even when there are no differences in feed intake between individuals. Richardson et al. (1996) reported a difference of approximately 1%

in dry matter digestibility between high RFI and low RFI growing bulls and heifers. In this case, the difference between the two groups in dry matter digestibility accounted for 14% of the variation in feed efficiency between high and low RFI animals (Richardson et al. 1996). These results are supported by those of Nkrumah et al. (2006), who found that low RFI steers possessed a 5% higher dry matter digestibility independent of dry matter intake.

2.6. Other production considerations

Increased feed efficiency seems to be linked to lower activity levels in cattle (Pitchford et al. 2004). However, it is currently unclear whether reduced activity levels are related to lower stress susceptibility, or merely the result of a lower ability to cope with stressors (Pitchford et al. 2004). In addition, there has been a report (Wassmuth et al. 2000) of improved feed efficiency being correlated (r=-0.24) with an increased incidence of "other" diseases in Danish Friesian dairy bulls. However, there was no mention of the severity or type of these "other" diseases. While these reports project a cautionary slant upon selecting for increased feed efficiency in cattle, they have not been substantiated by other studies, and therefore these few studies cannot be given a disproportionate amount of credibility.

The majority of research dealing with RFI has involved growing steers. The results obtained from these studies may be misleading, as the greatest portion of feed costs are incurred to maintain the mature cow herd, which consumes 50% of the total energy required for beef production (Montano-Bermudez et al. 1990). Preliminary results (Herd et al. 1998) demonstrated a 7% gain in the body weight of mature cows

ranked according to postweaning RFI with no concurrent increase in feed consumption, as well as a 15% numeric advantage (p=0.07) of low RFI cows when efficiency was expressed as calf body weight:cow feed intake. Archer et al. (2002) estimated phenotypic and genetic correlations between post-weaning RFI and mature cow RFI of r=0.40 and r=0.98, respectively, however; the diet used in that study was pelleted ration fed *ad libitum*, which is an unrealistic production scenario for any mature cow herd. A recent report (Arthur et al. 2005) compared various maternal traits, including pregnancy rate, calving rate, weaning rate, milk yield, and birth weight between mature cow lines selected for high and low RFI values over 5 years. The divergent selection for RFI did not seem to alter any maternal productivity traits (Arthur et al. 2005), although it is not entirely certain that significant differences in feed efficiency remained as the cows matured, as feed intake was not measured past the post-weaning period. Basarab et al. (unpublished data) demonstrated a significant difference in RFI and feed intake in mature cows when they were fed an *ad libitum* barley straw-silage diet, however, these cows were not evaluated for RFI value post-weaning. Clearly, more study is needed on the relationship between post-weaning and mature animal RFI in production situations, to ensure that selection based upon post-weaning RFI is appropriate and beneficial for mature as well as growing cattle.

While most reports do not note significant differences between efficient and inefficient animals in terms of meat quality (Maltin et al. 2001; Sinclair et al. 2001), there appears to be a slight correlation (r=0.17) between RFI and overall fatness of the animal (Arthur et al. 2004). A trend is emerging which implies that growing animals with low RFI tend to be leaner. Basarab et al. (2003) reported that high RFI and low RFI steers

differed (p=0.005) in empty body fat gain, with low RFI steers having a slower rate of empty body fat deposition. Although low RFI steers tended to be leaner (not statistically significant) in the Basarab et al. (2003) study, the main difference in fat deposition between high and low RFI groups arose in amount of body cavity fat, which is trimmed from the carcass. No difference was found in marbling score between high and low RFI groups (Basarab et al. 2003), which indicates no difference in meat quality and palatability to the consumer. Similar results in growing animals are reported in reviews by Archer et al. (1999), and Arthur et al. (2004).

The results reported by Arthur et al. (2004) and Basarab et al. (2003) are contradicted by those of Dicostanzo et al. (1991) who reported that efficient mature cows tended to be fatter. The discrepancy may arise from the fact that Dicostanzo et al. (1991) studied mature cows, while the other studies dealt with young, growing animals. It is possible that there are differences in the pattern of fat and protein deposition between mature and growing animals. Furthermore, since fat accretion acts as an insulator, it stands to reason that animals with a higher carcass fat content would expend less energy for thermogenesis, and therefore be more efficient. Since mature animals are not increasing muscle mass in the same manner as growing animals, it is possible that less energy is needed for protein synthesis, while more energy is needed to support thermogenesis in a larger body mass. This would provide fatter, mature cows with the advantage in feed efficiency. Basarab et al. (unpublished data) demonstrated that dams that produced low RFI calves exhibited 2-3 mm more backfat throughout the year than dams that produced high RFI calves. In addition, dams that produced high RFI calves lost more weight from pre-calving to pre-breeding (Basarab et al., unpublished data).

Alternatively, Arthur et al. (2005) reported that high RFI cows had a propensity to be fatter at the beginning of the mating season than low RFI cows, although fatness levels between the two groups were very comparable during the rest of the year.

This discrepancy is slightly troubling, although more research is needed in mature animals to confirm the results of Dicostanzo et al. (1991), Basarab et al. (unpublished data), and/or Arthur et al. (2005). If the most efficient young, growing cattle are leaner, will they continue that trend of leanness as mature animals? If that is the case, will they lose their advantage in feed efficiency as mature animals due to the increased leanness? Will the trend of increasing leanness eventually impact fertility over generations of selection? As most feed in a commercial operation generally goes to maintain the mature cow herd (Pitchford 2004), the above questions need to be addressed so that the benefits of improved feed efficiency in growing cattle do not vanish when the replacements reach maturity.

2.7. Genetic components of residual feed intake

It has been established (Arthur et al. 2001, van der Westhuizen 2004, Robinson and Oddy 2004) that RFI is regulated, at least in part, by a genetic component. It appears that there is greater variation in genetic RFI between individual animals, lending credence to the theory that divergent RFI values are due to the disparity in individual maintenance energy requirements, and not breed differences (Nkrumah et al. 2004). While attempts have been made to quantify the genetic component of RFI through quantitative trait loci (QTL) analysis (Pitchford et al. 2002; Nkrumah 2006), the underlying genetic mechanisms regulating feed efficiency are still relatively unclear. As

implementing a measurement system for RFI is expensive and time consuming (Archer et al. 1999), research has begun to focus on other means of determining feed efficient cattle using traits strongly correlated with RFI.

2.7.1. Insulin-like growth factor-1 (IGF-1)

Insulin-like growth factor-1 (IGF-1), a hormone involved with many aspects of growth and metabolism, has emerged as a likely candidate for the prediction of RFI (Johnston et al. 2002). Evidence supports the heritability of IGF-1 (h^2=0.34, 0.43) as well as a genetic correlation between circulating levels of plasma IGF-1 and RFI (r=0.39, 0.56) over two Australian datasets (Johnston et al. 2002). Moore (2003) also reported a slightly higher heritability (h^2=0.50) of IGF-1, and a very similar genetic correlation between IGF-1 and RFI (r=0.31), although the standard error was quite high (SE=0.36). The potential for using plasma IGF-1 concentration as an indicator for predicting RFI value led to a modelling scenario where the economic benefit of IGF-1 selection was investigated (Wood et al. 2004). This scenario indicated a profit of $3.30 (Japanese market) and $1.57 (domestic/Australian market) per head over the base scenario (no testing) when plasma IGF-1 concentrations were measured on both males and females at weaning. While plasma IGF-1 concentration may prove to be an adequate predictor of RFI, more research is needed, as the underlying physiological and/or genetic mechanisms driving the differences in IGF-1 concentration between individuals remain unclear.

2.7.2. *Review of microarray technology and its potential in residual feed intake (RFI) research*

Microarray technology is based upon the hybridization of fluorescently-labelled target complimentary DNA (cDNA) samples (reverse-transcribed from RNA) to probes of interest fixed on a solid surface, usually a specialized glass slide. (Walsh and Henderson, 2004). Probes can be expressed-sequence tags, cDNA clones, or synthesized oligionucleotides for genes of interest (Allison et al. 2006). The intensity of fluorescence of the hybridized probes is then used to estimate gene expression levels, as fluorescence will be greater with a larger number of transcripts present in the target sample (Allison et al. 2006). Frequently, a green-fluorescing dye (Cy3) is assigned to one treatment, while a red-fluorescing dye (Cy5) is assigned to a different treatment. The dye-treatment designation will then be reversed in a dye-swap procedure involving two microarray slides. The cDNA from one treatment will fluoresce green, the cDNA from the other treatment will fluoresce red, and yellow will be observed if cDNA from both treatments is present in roughly equal amounts (Walsh and Henderson 2004).

The advantage of microarray technology in livestock genomics research is that it facilitates the investigation of thousands of genetic elements at a time. This allows for a widespread analysis of genes that regulate economically important traits in beef cattle (Lehnert et al. 2006). As more knowledge is accumulated about the molecular and physiological mechanisms influencing beef cattle production, the manipulation of desirable traits will lead to further environmental, production, and economic benefits.

2.8. Conclusions

It is clear that selection for animals with favourable RFI values could be advantageous in decreasing feed costs for producers. This is especially true when one takes into account that selection for negative RFI (increased efficiency) does not seem to have an effect upon growth rate, finished weight, or mature size. The moderate heritability of RFI suggests that using measurements of RFI as a tool to increase feed efficiency is a definite possibility.

Many different metabolic processes in the body require energy. The ability of some cattle to perform or maintain these processes at a lower energetic cost allows them to use the excess energy for productive purposes, therefore requiring less feed. Protein turnover is an energetically expensive process that may account for differences between animals in feed consumption (efficiency). As both skeletal muscle and the GIT are constantly undergoing protein turnover, it seems most likely that variation in the energetic cost of protein turnover would occur in these two systems. If an absolute relationship can be defined between RFI and protein turnover (either in skeletal muscle or the GIT), the possibility exists to develop selection criteria to identify animals with a low energy requirement for protein metabolism, which would then be more feed efficient. Additionally, the ruminant GIT is especially complicated in modes of digestion and nutrient absorption, potentially allowing for large variation between individuals in these processes – especially considering the existing evidence with regards to starch digestion and methane production.

With recent advances in molecular biology to facilitate genetic analysis of cattle in relation to RFI, it is clear that genetic deviations play a role in feed efficiency. Further

discovery and fine mapping of QTLs as well as more detailed microarray analyses could lead to a more comprehensive understanding of the underlying genetic mechanisms contributing to differences in RFI. Molecular markers such as IGF-1, or another entity that is currently undefined, could make distinguishing between high and low RFI animals both economical and practical.

The link between increased leanness and improved feed efficiency in growing animals is cause for slight concern, as a certain level of fatness is desirable for reproduction as well as meat quality, and should be investigated more thoroughly to ensure a minimal additive effect over time. As well, supplementary research should be conducted on mature animals to determine whether the advantage in RFI in growing animals is continued in the mature cow herd, and over generations. Regardless, it appears that measures of RFI in beef cattle could be a very promising method of increasing producer profit through improved feed efficiency.

Table 2.1. Literature estimates of heritability of residual feed intake in growing cattle.

Heritabilty	Source
0.28 ± 0.11	Koch et al. (1963)
0.14 ± 0.12	Fan et al. (1995)
0.44 ± .07	Arthur et al. (1997)
0.27 ± .23	Brelin and Brannang (1982)
0.22 ± 0.11	Korver et al. (1991)

Adapted from Archer et al. (1999).

Table 2.2. Effect of cattle breed on 3-methylhistidine (3-MeH) urinary elimination at similar rates of liveweight gain.

	3-MeH urinary elimination (μmol/kg body weight/d)	
Breed	1 kg/d liveweight gain	1.2 kg/d liveweight gain
Charolais	2.146	2.267
Aberdeen Angus	1.0805	2.172
Effect of intake	$p < .001$	
Effect of breed	$p < .001$	

Adapted from Lobley (1998).

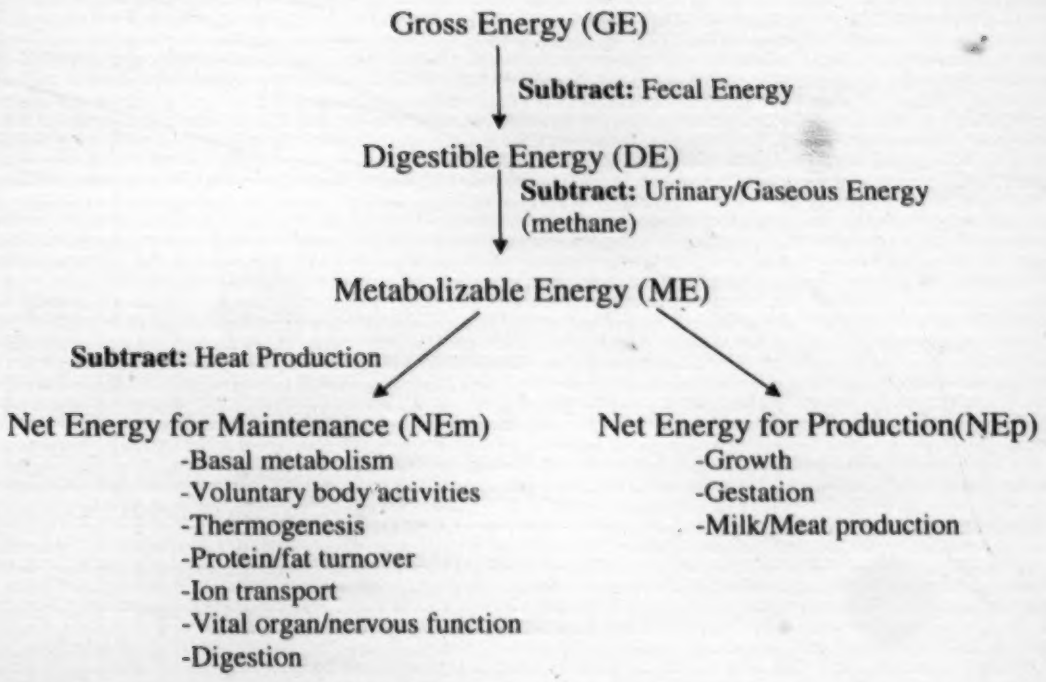

Figure 2.1. Energy partitioning in cattle. Adapted from Baldwin and Sainz (1995).

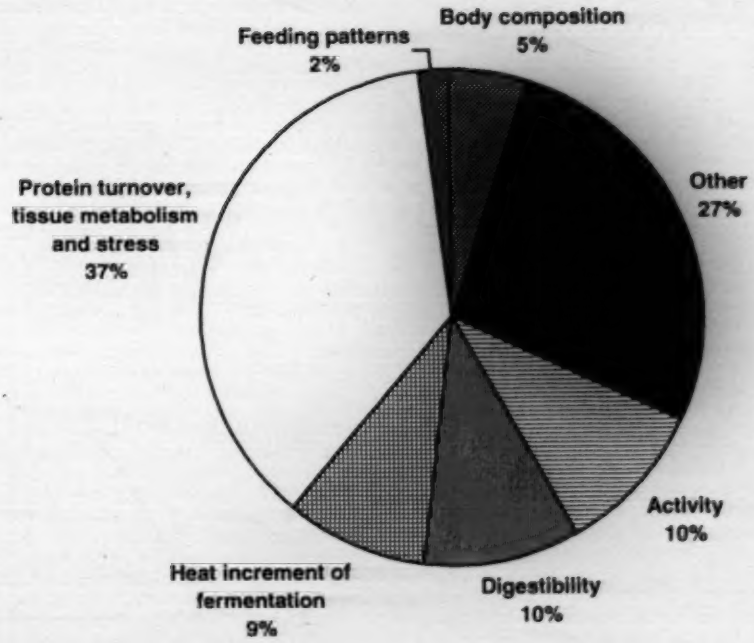

Figure 2.2. Contributions of biological mechanisms to variation in residual feed intake as determined from experiments on divergently selected cattle.
Adapted from Richardson and Herd (2004).

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Chapter 3

Energy expenditure and protein degradation as contributors to variation in residual feed intake (RFI) of growing beef steers

3.1. Introduction

The profit margin in the beef industry is highly dependent upon input costs, most notably that of feed. Consequently, the productive efficiency of cattle in converting feed energy to growth and production is of the greatest interest to producers. Residual feed intake (RFI), which is the difference between an animal's actual feed intake and expected feed intake (based upon body weight and growth rate), is a measure of feed efficiency that has considerable potential to decrease feed costs (Archer et al. 1999). Efficient animals consume less feed than is expected, and are denoted with a negative value, whereas inefficient animals consume more than is expected and are represented with a positive value.

It has repeatedly been illustrated that both phenotypic and genetic variation exists between individuals in RFI (Archer et al. 1999; Arthur et al. 2001; van der Westhuizen et al. 2004), indicating that there is potential for selection and improvement in beef cattle feed efficiency based upon RFI. Many of the biological processes that contribute in some manner to the variation between individuals in RFI have been elucidated and include, but are not limited to, body composition, feeding patterns, protein turnover/tissue metabolism, heat increment of feeding, digestibility of diet, activity, and "other" unknown factors (DiCostanzo et al. 1990; Johnson et al. 2003; Herd et al. 2004). The complexity lies in ascertaining the underlying factors that cause these biological processes to fluctuate among individuals.

The partitioning of dietary energy into various metabolic processes may play a role in altering RFI in beef cattle. As gross feed energy passes through the digestive system, various losses occur in the form of fecal, urinary, gaseous (methane), and heat energy, leaving a residual portion (net energy) for maintenance of bodily functions and productive purposes (Baldwin and Sainz 1995). Deviations from the normal pattern of energy partitioning could very well lead to variation between animals in feed efficiency. Basarab et al. (2003) observed decreased liver, stomach, and intestine weights in low RFI (efficient steers), as well as decreased maintenance energy requirements. The reduced maintenance energy requirements in low RFI steers may be a reflection of decreased energy required for protein turnover to maintain cell structure and function in the smaller visceral organs of the low RFI steers. Generally estimated to contain 5.5-6.5% of gross energy intake (Gibbs and Leng 1993), methane production is considerably variable between low and high RFI steers. Herd et al. (2002) and Nkrumah et al. (2006) both report a significantly higher methane production in high RFI steers. In addition, differences between animals in protein turnover (Richardson and Herd 2004) and protein versus fat deposition (Basarab et al. 2003) impacts RFI to some extent.

The scientific evidence available clearly implies distinct animal variation in energy partitioning, especially with regards to visceral organ metabolism, methane production, heat production, and protein/fat metabolism in beef cattle. Improved knowledge about the exact mechanisms responsible for the variation in energy partitioning between animals will allow for more informed selection decisions by producers to maximize profitability. The present study examined methane production,

heat production, and skeletal muscle metabolism in growing beef steers with divergent RFI values.

3.2. Materials and methods

3.2.1. Animals and diets

Eighty-one steers were assessed in a feedlot situation for feed efficiency using the GrowSafe™ automated feeding system (GrowSafe™ Systems Ltd., Airdrie, Alberta) at the University of Alberta Research Station (Kinsella, Alberta) for 95 d, complying with the accepted minimum time of 63 d on test to accurately determine RFI of (Wang et al. 2006). Animals were ranked based upon RFI value, and eight steers were selected (three low RFI, five high RFI; Table 3.1) to undergo further experimentation at the University of Alberta's Metabolic Research Station (Edmonton, Alberta). Steers were fed the same diet for both the feedlot and metabolic trials, which contained 64.5% barley, 20% oats, 9% alfalfa hay pellets, 5% supplement, and 1.5% canola oil (Table 3.2).

Selected steers were halter-trained before arrival at the Metabolic Research Station in Edmonton, where they were housed indoors in individual pens. The environment was temperature controlled with plentiful bedding and water at all times. The steers were acclimated to their new surroundings, gradually brought up to the required feeding level, and slowly adapted to confined respiration calorimetry stanchions and metabolism crates during an adjustment period of 14 d. Low RFI steers had a body weight of 541.67 ± 55.73 kg, while high RFI steers weighed 531.40 ± 17.00 kg at the commencement of the trial. Feeding level was determined using the National Research Council (NRC 1996) maintenance requirement of 0.077 Mcal NEm/$BW^{0.75}$ and

multiplying by either 1.2 for maintenance level, or 2.5 for full feed. All steers in the study were cared for according to the guidelines set out by the Canadian Council on Animal Care (CCAC 1993).

3.2.2. Indirect calorimetry

Methane production and oxygen consumption were measured in an open circuit, indirect calorimetry system with four chambers (Delfino et al. 1988). After acclimation, animals were randomly situated in one of four stanchions. Stanchions were designed to accommodate standing and lying down, as well as free movement of the head in a vertical direction. The steers' heads were placed in canvas hoods attached to small chambers where feed and water were located, and the hoods were firmly fastened to prevent oxygen/methane leakage.

Respired air was passed through Drierite (W.A. Hammond Drierite Co. Ltd., Xenia, Ohio), removing excess water vapour, and was analyzed for methane (Model 880A Infrared Analyzer, Rosemount Analytical, Orville, Ohio) and oxygen content (Servomex Inc., Sussex, UK). Airflow rate (Foxboro 823 IFO integral flow orifice with cell transmitter, Invensys Systems Inc., Foxboro, MA) and pressure (Foxboro 821AL absolute pressure transmitter, Invensys Systems Inc., Foxboro, MA) were also measured. The system was calibrated by the nitrogen injection method previously described by Young et al. (1984).

Each steer underwent two 16 h non-consecutive measurements in the calorimetry system at both the 2.5 X and 1.2 X maintenance feeding levels. The calorimetry measurements completed at each feeding level occurred at least 2d apart, and

approximately one month lapsed before calorimetry measurements were performed at the 1.2 X maintenance feeding level. This was to allow for acclimation to the reduced feed level and to ensure that no residual effect on methane and oxygen production remained from the 2.5 X feeding level.

Methane production was measured in litres per day and heat production was calculated as (-4.90 Kcal/L oxygen)*(volume of expired air at STP)*(oxygen in exhaust air – oxygen in inlet air at STP) (McLean and Tobin 1990). Heat production as a function of metabolic weight, methane production as a function of metabolic weight, dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) were all analyzed as dependent variables using repeated measures in PROC MIXED of SAS, with RFI group (high or low) as the independent variable, and animal as the repeated measure. The random factor included in the model was the calorimetry chamber (labelled 1-4). Correlations between heat production, methane production, DMI, ADG, and FCR were performed using PROC CORR in SAS (SAS Institute, Inc., North Carolina, USA).

3.2.3. Urine collection

Steers were housed in individual metabolic crates to facilitate the collection of urine. Crates allowed for standing and lying positions, and steers were supplied with both feed and water at regular intervals. Total (pre-acidified) urine was collected over a 5 d period for both the 1.2 X and 2.5 X maintenance feeding levels. Approximately 2% of total urine was sub-sampled (after mixing) per animal per day and stored at -20°C for later analysis.

Daily urine samples were pooled for each animal before undergoing high performance liquid chromatography (HPLC) analysis to determine the concentration of 3-methylhistidine (3-MeH). Samples were thawed in a water bath and prepared as follows. The internal standard, 0.1 mL histidinol (100 x 10^6 M) was added to 0.2 mL of urine. Samples were deproteinized with 0.1 mL $HClO_4$ (3 M) and centrifuged at 3000 RPM for 15 min. Then 0.2 mL H_2O, 0.25 mL NaOH (1.5 M), and 0.25 mL $Na_2B_4O_7$ (pH 9.0) were added to the deproteinized samples. The samples were vortexed while adding 0.25 mL fluorescamine (160 mg/ 100 mL acetonitrile). They were then incubated for 3-4 seconds at room temperature, after which 0.25 mL HCl (2 M) was added. Samples were capped, mixed, and incubated for 45 min at 90°C in a water bath. After incubation, samples were extracted twice with 1.5 mL of diethyl ether. Samples were analyzed using a Varian Model 5500 Liquid Chromatograph (Varian Inc., Palo Alto, CA) with a Varian 2070 spectrofluorometer detector (Varian Inc., Palo Alto, CA) and a Varian 9090 auto analyzer (Varian Inc., Palo Alto, CA). The separations were completed on a 3 micron reverse phase column (Supelcosil 4.6 x 150 mm C18 column) using a binary gradient as follows: Solvent A consisted of 2.5 mM cetyltrimethylammonium bromide and 0.1 M sodium acetate buffer (pH 6.5), while Solvent B contained 10% (v/v) 2.5 mM cetyltrimethylammonium bromide and 90% (v/v) acetonitrile (pH 6.5). The flow rate was 1 mL/min.

The data were analyzed using PROC MIXED in SAS. The model involved total 3-MeH production (Tot 3-MeH) and 3-MeH production expressed as a function of metabolic weight (Met 3-MeH) as dependent variables, with RFI group (high or low) and the interaction between RFI group and day of sampling as the independent variables. The

random factor in the model was RFI group nested within animal. Correlations between RFI group, Tot 3-MeH, and Met 3-MeH were performed using PROC CORR in SAS (SAS Institute, Inc., North Carolina, USA).

3.3. Results

3.3.1. Indirect calorimetry

Due to a possible mechanical malfunction of the methane analyzer during the calorimetry measurements at the 1.2 X maintenance feeding level, the results for methane production at that feeding level will not be reported here. The methane measurements at the 2.5 X maintenance feeding level as well as the other metabolic measurements at the 1.2 X maintenance feeding level were not affected. Heat production, methane production (2.5 X maintenance only), DMI, ADG, and FCR values for each feeding level are shown in Table 3.3 and 3.4. There was a significant difference between high and low RFI groups in FCR ($p<0.0001$) at the 1.2 X maintenance feeding level, with the low RFI group demonstrating a lower FCR. The effect of RFI group upon FCR ($p<0.0001$) remained at the 2.5 X maintenance feeding level.

The statistically significant correlations between heat production, methane production (2.5 X maintenance only), DMI, ADG, and FCR are listed in Table 3.5 and 3.6. At the 1.2 X maintenance feeding level, residual feed intake and ADG exhibited a negative relationship ($r=-0.4878$, $p<0.0001$), as did FCR and ADG ($r=-0.6977$, $p<0.0001$). Positive correlations existed between RFI and FCR ($r=0.91093$, $p<0.0001$), heat production and DMI ($r=0.3439$, $p=0.0018$), and DMI and ADG ($r=0.6579$, $p<0.0001$). At the 2.5 X maintenance feeding level, negative correlations were also

revealed between RFI and ADG (r=-0.4878, p<0.0001), heat production and methane production (r=-0.4730, p=0.0005), methane production and FCR (r=-0.3265, p=0.0207), DMI and FCR (r=-0.2231, p=0.0467), and ADG and FCR (r=-0.6978, p<0.0001). Positive correlations were present between RFI and FCR (r=0.9109, p<0.0001), heat production and DMI (r=0.3664, p=0.0008), heat production and ADG (r=0.2390, p=0.0328), methane production and ADG (r=0.2990, p=0.0349), and DMI and ADG (r=0.7460, p<0.0001).

3.3.2. 3-methylhistidine (3-MeH)

Consistent results for individual animals were not obtained due to a large daily variation in urine production at both feeding levels, although only the 2.5 X maintenance feeding level is shown (Table 3.7). There was a significant day of sampling effect (p=0.0422) upon Tot 3-MeH at the 2.5 X maintenance feeding level, and a trend (p=0.0722) at the 1.2 X maintenance feeding level, which illustrates the above point. The same effect persisted, although at a lower significance (p=0.0537 and p=0.0555, respectively), when Met 3-MeH results were analyzed. A trend (p=0.0696 and p=0.0823) also existed for Tot 3-MeH and Met 3-MeH to be affected by the RFI*sampling day interaction at the 2.5 X maintenance feeding level (Table 3.8). While not statistically significant, a positive numeric correlation was observed between RFI group, Tot 3-MeH and Met 3-MeH concentrations at both feeding levels.

3.4. Discussion

3.4.1. Indirect calorimetry

Although the calorimetry portion of this study was designed to lend further credence to the well accepted reports of increased methane production in high RFI animals, the lack of a statistically significant difference in methane production between RFI groups disagrees with many well-documented accounts to the contrary (Herd et al. 2002; Okine et al. 2003; Hegarty et al. 2004; Nkrumah et al. 2006). The most reasonable explanation for this discrepancy lies in the small sample population. This author does not believe that the results from this study, in terms of the interaction between RFI group and methane production, would be reproducible in a larger sample population. Similarly, while reports have stated considerable distinction in heat production between high and low RFI groups (Hotovy et al. 1991; Basarab et al. 2003), no significant interaction was seen in this study.

Nkrumah et al. (2006) reported positive correlations (r=0.68 and r=0.44) between RFI value, heat production, and methane production, respectively, which disagree with the results of this study. In addition, Nkrumah et al. (2006) reported significant positive correlations between heat production and FCR (r=0.37), as well as DMI and methane production (r=0.38), which were not seen in the present study. However, in accordance with the findings of Nkrumah et al. (2006), the relationship between DMI and heat production was very similar (r=0.31 versus r=0.37). Again, the failure of this study to support previous research upon the effect of RFI on heat and methane production was most likely due to the small sample size available for use in the present study. The facilities at the Metabolic Unit of the University of Alberta require that cattle on test be

haltered, and the second group of eight steers due to be tested were unable to be halter-trained to the degree necessary for safe handling.

3.4.2. 3-methylhistidine (3-MeH)

Previous studies have shown that the concentration of 3-methylhistidine excreted in bovine urine is a reliable measure of skeletal muscle degradation (Nishizawa et al. 1979; Harris and Milne 1981; McCarthy et al. 1983). This study failed to unearth any statistically significant differences between RFI groups in 3-MeH concentrations. This is largely because the amount of urine, and subsequently 3-MeH, was extremely variable over the 5 day sampling period. Therefore, it was very difficult to obtain consistent results from a population that did not have relatively similar urine outputs over the sampling period, as was the case in this experiment. Research in humans has demonstrated a coefficient of variation of 3-MeH production ranging from 2.2 to 7.0% (Lukaski et al. 1981). The coefficients of variation of Tot 3-MeH production reported in this study are considerably higher (34% and 49% at the 1.2 X maintenance and 2.5 X maintenance feeding levels, respectively), and remained almost identical for Met 3-MeH production. With such extreme irregularities in the quantity of 3-MeH excreted by the same animal during different days, it is impossible to reach any definite conclusions about the contribution of the rate of protein degradation to differences in RFI.

As environmental conditions in this study were unchanged day to day, the reasons for the highly variable 3-MeH output are unclear, although these differences may be reduced over a larger sample population. It is possible that the inherent stress of semi-restricted movement had an impact upon 3-MeH production, possibly explaining why

most (but not all) of the highest values in Table 3.6 are toward the end of the five day sampling period. The steers may have become more accustomed to their surroundings towards the end of the sampling period, resulting in more water consumed on a daily basis, as additional water intake will increase urinary output (Paquay et al. 1970). Stress also has an effect upon protein degradation (Richardson et al. 2004), therefore; if the steers were stressed in the metabolic crates, an increased rate of protein degradation could account for the higher Tot and Met 3-MeH values toward the end of the sampling period.

Previous researchers have speculated that protein turnover and ion transport energy loss could account for up to 67% of the variation between individuals in RFI (Herd et al. 2004), and have determined that more feed inefficient steers have a higher rate of protein degradation (Oddy et al. 1998). Richardson et al. (2004) reported no significant difference between low and high RFI steers in urinary 3-MeH concentration, although they postulate that stress and body composition (low RFI steers were leaner) may have been contributing factors. While this study is in agreement with the results of Richardson et al. (2004), it does not shed further light upon the effect of muscle protein degradation upon feed efficiency. It seems unlikely that protein metabolism is completely unrelated to RFI and feed efficiency, given that 15-20% of the energy cost of basal metabolism is attributed to protein synthesis alone (Waterlow 1984). In addition, as protein synthesis requires approximately five times more energy than does protein degradation (Lobley et al. 2000) a small change in the amount of protein synthesis would have a greater effect on energy utilization than a small change in the amount of protein degradation. It is credible that an examination of protein synthesis may reveal a more notable connection with RFI.

Table 3.1. RFI values of animals utilized in indirect calorimetry and 3-methylhistidine (3-MeH) analysis.

Animal	RFI Value
225	2.15
143	1.53
239	1.41
241	1.35
253	1.31
87	-1.06
47	-1.29
163	-1.49

Table 3.2. Ingredient composition of animal diet.

Ingredient	% as fed basis
Barley	64.50
Oats	20.00
Alfalfa hay pellets	9.00
Supplement[1]	5.00
Canola Oil	1.50

[1]Consisted of: 5.5% Ca, 0.28% P, 0.64% K, 1.98% Na, 0.15% S, 0.31% Mg, 16 mg/kg I, 28 mg/kg Fe, 1.6 mg/kg Se, 160 mg/kg Cu, 432 mg/kg Mn, 432 mg/kg Zn, 4.2 mg/kg Co, 440 mg/kg monensin, 80 000 IU/kg vitamin A, 8 000 IU/kg vitamin D, and 1 111 IU/kg vitamin E.

Table 3.3. Heat production, methane production, dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) values of residual feed intake groups[1] at 1.2 X maintenance feeding level.

Trait	High RFI	Low RFI	P-value
Heat Production (kcal/kg$^{0.75}$)	120.86±1.76	121.21±2.27	0.9056
DMI (kg)	4.66±0.066	4.720.085	0.5643
ADG (kg/d)	1.63±0.060	1.80±0.078	0.1174
FCR	7.67±0.21	5.52±0.28	<0.0001

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d

Table 3.4. Heat production, methane production, dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) values of residual feed intake groups[1] at 2.5 X maintenance feeding level.

Trait	High RFI	Low RFI	P-value
Heat Production (kcal/kg$^{0.75}$)	141.88±2.62	141.99±3.38	0.9804
Methane Production (L/kg$^{0.75}$)	2377.61±125.95	2459.17±125.95	0.6609
DMI (kg)	10.30±0.20	10.44±0.26	0.7021
ADG (kg/d)	1.63±0.060	1.80±0.078	0.1174
FCR	7.67±0.21	5.52±0.28	<0.0001

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d

Table 3.5. Pearson correlation coefficients of the relationships between residual feed intake (RFI) value, heat production, methane production, dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) for 1.2 X maintenance feeding level.

	RFI Value[1]	Heat Production ($kcal/kg^{0.75}$)[2]	DMI	ADG	FCR
RFI Value[1]	1.00	-0.153	-0.201*	-0.488***	0.911***
Heat Production ($kcal/kg^{0.75}$)[2]	-0.153	1.00	0.344***	0.125	-0.145
DMI	-0.201*	0.344***	1.00	0.658***	-0.204*
ADG	-0.488***	0.125	0.658***	1.00	-0.698***
FCR	0.911***	-0.145	-0.204*	-0.698***	1.00

*$p \leq 0.10$
**$p \leq 0.05$
***$p \leq 0.01$
[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]Expressed as a function of metabolic body weight

Table 3.6. Pearson correlation coefficients of the relationships between residual feed intake (RFI) value, heat production, methane production, dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) for 2.5 X maintenance feeding level.

	RFI Value[1]	Heat Production (kcal/kg$^{0.75}$)[2]	Methane Production (L/kg$^{0.75}$)[2]	DMI	ADG	FCR
RFI Value[1]	1.00	-0.034	-0.217	-0.152	-0.488***	0.911***
Heat Production (kcal/kg$^{0.75}$)[2]	-0.034	1.00	-0.473***	0.366***	0.239**	0.022
Methane Production (L/kg$^{0.75}$)[2]	-0.217	-0.473***	1.00	-0.064	0.299**	-0.326**
DMI	-0.152	0.366***	-0.064	1.00	0.746***	-0.223**
ADG	-0.488***	0.239**	0.299**	0.746***	1.00	-0.698***
FCR	0.911***	0.022	-0.326**	-0.223**	-0.698***	1.00

*$p \leq 0.10$
**$p \leq 0.05$
***$p \leq 0.01$
[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]Expressed as a function of metabolic body weight

Table 3.7. Urinary 3-methylhistidine (3-MeH) concentration in high and low residual feed intake (RFI) groups[1] over a 5 d sampling period at 2.5 X maintenance feeding level.

Animal	RFI Group	Day	Tot 3-MeH[2] (nmol/L)	Met 3-MeH[3] (nmol/kg$^{0.75}$)
225	High	1	569.92	5.32
	High	2	2149.33	20.06
	High	3	997.78	9.31
	High	4	1105.03	10.31
	High	5	1236.39	11.54
143	High	1	1518.07	13.50
	High	2	584.70	5.20
	High	3	230.55	2.05
	High	4	568.40	5.05
	High	5	951.11	8.46
239	High	1	798.13	7.02
	High	2	592.60	5.21
	High	3	573.26	5.04
	High	4	490.95	4.32
	High	5	1067.34	9.39
241	High	1	544.17	4.90
	High	2	798.33	7.19
	High	3	972.27	8.75
	High	4	786.97	7.08
	High	5	1416.92	12.76
253	High	1	1230.68	11.30
	High	2	892.79	8.20
	High	3	1360.60	12.49
	High	4	1262.75	11.60
	High	5	385.77	3.54

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]Total amount of 3-methylhistidine produced/d in nmol/L
[3]Amount of 3-methylhistidine produced/d in nmol/kg, expressed as a function of metabolic body weight

Table 3.7 (con't). Urinary 3-methylhistidine (3-MeH) concentration in high and low residual feed intake (RFI) groups[1] over a 5 d sampling period at 2.5 X maintenance feeding level.

Animal	RFI Group	Day	Tot 3-MeH[2] (nmol/L)	Met 3-MeH[3] (nmol/kg$^{0.75}$)
87	Low	1	1155.02	10.79
	Low	2	504.64	4.71
	Low	3	1167.95	10.92
	Low	4	548.56	5.13
	Low	5	658.14	6.15
47	Low	1	650.69	5.33
	Low	2	560.07	4.55
	Low	3	1010.98	8.28
	Low	4	1194.00	9.78
	Low	5	1506.32	12.33
163	Low	1	197.46	1.84
	Low	2	469.16	4.37
	Low	3	1127.15	10.49
	Low	4	992.91	9.24
	Low	5	1978.00	18.40

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]Total amount of 3-methylhistidine produced/d in nmol/L
[3]Amount of 3-methylhistidine produced/d in nmol/kg, expressed as a function of metabolic body weight

Table 3.8. Effect of residual feed intake (RFI) group[1], day and RFI group*day interaction upon urinary 3-methylhistidine (3-MeH) concentration.

Feeding Level	3-MeH Measurement	High RFI	Low RFI	Effect	P-value
2.5 X Maintenance	Tot 3-MeH[2]	951.77±82.95	916.96±107.13	RFI Group	0.8060
				Day	0.0422
				RFI*Day	0.0696
	Met 3-MeH[3]	8.64±0.81	8.17±1.05	RFI Group	0.7352
				Day	0.0537
				RFI*Day	0.0823
1.2 X Maintenance	Tot 3-MeH	1399.99±125.80	1523.42±162.41	RFI Group	0.5699
				Day	0.0722
				RFI*Day	0.5309
	Met 3-MeH	12.67±1.26	13.70±1.63	RFI Group	0.6362
				Day	0.0555
				RFI*Day	0.5188

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]Total amount of 3-methylhistidine produced/d in nmol/L
[3]Amount of 3-methylhistidine produced/d in nmol/kg, expressed as a function of metabolic body weight

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Chapter 4

Microarray analysis of genetic components potentially related to residual feed intake (RFI) in growing beef steers

4.1. Introduction

Measures of feed efficiency in beef cattle, most notably residual feed intake (RFI), have been identified as promising ways to reduce the input cost of feed in production situations. Reducing the amount of feed consumed by the animal without sacrificing growth rate leads to substantial gains in profitability and market competitiveness (Herd et al. 2003). Recently, research efforts have shifted from a production-based mindset to focus more heavily on the underlying mechanisms regulating growth and energy usage in cattle. Advances in the use and understanding of molecular technologies have permitted the examination of various genetic components of beef production. Quantitative trait loci (QTL) studies of production (Casas et al. 2000; Kneeland et al. 2004), carcass merit (Casas et al. 2000; Moore et al. 2003; Li et al. 2004), and more recently, RFI (Hoque et al. 2005; Nkrumah 2006) have made considerable progress towards hastening genetic improvement in beef cattle. Still, with all the advantages acquired with the efforts mentioned above, a clear mechanism for the selection of more feed efficient cattle is still lacking.

Measuring feed efficiency in a population is quite time-consuming and expensive (Archer et al. 1999), and therefore quite impractical for large-scale implementation. However, there is potential for circulating insulin-like growth factor-1 (IGF-1) concentration in the blood to predict (at least to some extent) RFI in cattle (Johnston et al. 2002; Wood et al. 2002). Although blood collection is more cost-effective compared to

RFI testing itself, it still requires intensive handling of the cattle tested, as well as thorough knowledge of the proper collection methodology to avoid potential injury and stress to the animal. If a suitable candidate gene for the accurate prediction of RFI is identified, producers could acquire a hair sample for analysis, a process that is quite straightforward and uncomplicated. In addition, hair samples are already obtained from many breeding animals for karyotyping purposes, and perhaps the two tests could be combined for ease of handling and cost-effectiveness.

Microarray studies have received a great deal of attention for their ability to analyze thousands of genetic elements on a single array. A great deal of useful information can be gleaned from a microarray experiment; however, it is necessary to ensure that adequate experimental design and statistical analysis measures are in place to guarantee the validity of the results (Churchill 2002). Thus far, gene interaction and regulatory information in beef cattle provided by microarray research has been limited to the investigation of disease (Wilson et al. 2005), skeletal muscle (Reverter et al. 2005), weight loss (Byrne et al. 2005), and *in vitro* adipogenesis (Tan et al. 2006). The present study attempted to identify preliminary differentially expressed candidate genes in cattle exhibiting divergent RFI values, with the assistance of microarray technology. To this author's knowledge, this is the first study to combine RFI and microarray data in beef cattle.

4.2. Materials and methods

4.2.1. Animals and tissue collection

Thirteen composite beef steers were previously evaluated for RFI in a feedlot situation at the Lacombe Research Centre (Lacombe, Alberta) by Dr. John Basarab and his colleagues, who kindly permitted tissue sampling at slaughter. Residual feed intake (RFI) values can be found in Table 4.1. Two steers (I.D. P1081 and P1065) were later omitted from the study due to RFI values very near zero. Immediately after the removal of the gastrointestinal tract (GIT) from the body cavity during the slaughter process, a rumen tissue sample was removed from the rumen wall of each steer. Samples were thoroughly washed in sterilized water to remove latent digesta present in the rumen at the time of slaughter, placed in a 50 mL sterile screw top container, snap-frozen in liquid nitrogen, and placed in a cooler filled with dry ice for transport back to the University of Alberta, where they were stored at -80°C until further analysis.

4.2.2. RNA extraction from rumen tissue

Approximately 1g of rumen tissue was ground with a mortal and pestle and transferred into two 14 mL Falcon tubes (approximately 0.5 g tissue/tube). RNA from the rumen tissue samples was extracted using TRIzol methodology (Chomczynski and Sacchi 1987) (Appendix 1). To each tube, 5 mL TRIzol was added, and samples were homogenized for 5-10 s on ice. Samples were then incubated at room temperature for 20 min and centrifuged for 10 min. The supernatant was removed to new tubes, 1 mL chloroform was added, samples were vortexed, and then incubated at room temperature for 2-3 min. These samples were centrifuged again for 15 min, and the supernatant was

transferred to new tubes. 1.25 mL of isopropanol alcohol and 1.25 mL of high salt solution were added, samples were vortexed, and incubated at room temperature for 15 min. Samples were centrifuged for 10 min, the supernatant was removed, the resultant pellet washed with 5 mL 75% (v/v) ethanol, and then centrifuged for 3 min at 8300 RPM. Again, the supernatant was removed, and the pellet was solubilized in 300 µL DEPC treated water.

4.2.3. Sample preparation for microarray

Epoxy microarray slides (Genetix, Hampshire, UK) were spotted with approximately 8700 individual bovine 70mer oligonucleotides in duplicate (Qiagen Operon Biotechnologies, Inc., Alabama, USA) using QArray 2 (Genetix, Hampshire, UK). RNA samples extracted from rumen tissue were prepared according to the protocol accompanying the Amino Allyl MessageAmp™ II aRNA Amplification Kit, available from www.ambion.com. Briefly, RNA was reverse transcribed to first strand complimentary DNA (cDNA). Then second strand cDNA was synthesized. The cDNA was purified and amino allyl-modified aRNA was transcribed. The aRNA was purified and then quantified using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Delaware, USA). The aRNA was coupled with Cy3 and Cy5 dyes, purified, and 70 µL of labelled aRNA was hybridized to the microarray slide for 16-20 h. Slides were washed with a low, high, and final stringency wash and dried before scanning. The detailed protocol is available in Appendix 2.

4.2.4. *Microarray analysis*

A pooled reference design was implemented (Kerr and Churchill 2001), wherein individual samples from each RFI group (high and low) were dye-swapped with a pooled sample containing all the individuals from the opposite RFI group. Slides were scanned with AQuire (Genetix, Hampshire, UK), and the resulting spots were analyzed with Genetix QScan (Genetix, Hampshire, UK). The spot finding feature of QScan was found to be slightly inadequate, so spot size and location was adjusted manually to determine the most precise intensities. Cy3 and Cy5 intensities were transferred from the QScan to an Excel file where they were $\log_2$ transformed, following the reasoning of Reverter et al. (2003). Global normalization and individual gene analysis were performed using a mixed model in SAS (SAS Institute, Inc., North Carolina, USA). The fold change p-values were adjusted with the Bonferroni Correction, and correlations between the duplicate Cy5 and Cy3 intensities on each pair of slides were performed. Steer P3063 was removed from further study due to a correlation of <80% between duplicate Cy5 intensity values. Genes identified as significant ($p \leq 0.05$), with a threshold fold expression level of ≥ 1.5 for upregulated genes and ≤ -1.5 for downregulated genes, were further scrutinized as to their relationship with RFI.

4.2.5. *Validation of microarray results*

As microarrays have a propensity to reveal a high number of "false positives" due to the immense amount of data generated, it is necessary to independently validate the results revealed by the microarray study. Independent validation of microarray results can be accomplished through standard quantitative real-time polymerase chain reaction

(QRT-PCR) methodology (Chuaqui et al. 2002). In this study, QRT-PCR was performed according to standard TaqMan® methodology (Applied Biosystems, California, USA). To summarize, RNA samples are reverse transcribed to cDNA, combined with a forward primer, reverse primer, a highly specific fluorescent probe, an internal standard, and amplified (Appendix 3). The number of amplification cycles (CT) it takes for the product to pass a threshold value compared to the CT for the internal standard determines the upregulation or downregulation of the particular gene of interest in relation to the internal standard.

In this study, nine genes were chosen for validation via QRT-PCR. Amplification was performed in triplicate on the ABI Prism 7700 Sequence Detection System (Applied Biosystems, California, USA). Primers and probes for each gene can be found in Table 4.2 and 4.3, respectively. The genes of interest were chosen based upon the number of animals in each RFI group that demonstrated expression of said genes at a statistically significant level, as well as if the upregulation/downregulation phenomenon was present in the low RFI group as indicated by the microarray study. Primers were designed with PrimerExpress® software (Applied Biosystems, California, USA). Results were analyzed with the Sequence Detector v. 1.7 software (Applied Biosystems, California, USA). Upregulation or downregulation of each gene was determined by calculating the ΔCT value, which is the difference between the CT value of the internal standard and the CT value of the gene of interest. A high concentration of specific transcripts will result in faster amplification, and a lower CT value, indicating upregulation compared to the internal standard. A lower concentration of transcripts results in slower amplification and a higher CT value, signifying downregulation of the target gene. The internal standard

utilized in this study was 18S ribosomal RNA. Regression analysis of the QRT-PCR results for all 10 animals on RFI was performed using PROC REG in SAS (SAS Institute, Inc., North Carolina, USA). Significance was assessed at $p \leq 0.05$.

4.3. Results

Twenty-two genes were found to be differentially expressed at a statistically significant level in at least one animal in both the high and low RFI groups (Table 4.4). However, while all these genes were classified as downregulated in the high RFI group ($p \leq 0.05$), there were discrepancies in the low RFI group, with some individuals having those genes upregulated (as would be expected), and some individuals having the same genes downregulated ($p \leq 0.05$).

Due to the tendency of individual animals in the low RFI group to exhibit conflicting results with regards to the upregulation or downregulation of significantly expressed genes, nine genes (Table 4.2) were chosen to confirm or refute the microarray results via QRT-PCR. These "test" genes were chosen based upon the number of animals in both RFI groups expressing that particular gene at a statistically significant level, as well as whether or not the gene was wholly upregulated, downregulated, or a mixture of both in the low RFI group. QRT-PCR results are shown in Table 4.5. In general, the QRT-PCR results were in agreement with the microarray results, however, as QRT-PCR was performed on all 10 animals and not just the ones that demonstrated significant gene expression in the microarray study, it became clear that upregulation or downregulation of the same gene in a particular RFI group was inconsistent. This phenomenon was more pronounced in the low RFI group than the high.

Regression analysis of QRT-PCR results against RFI value illustrated a significant relationship between SH3 domain-binding glutamic acid-rich protein like 3 (SH3BGRL3) and RFI with a regression coefficient of −1.08 (p=0.0083), with RFI value explaining 65% of the variation in SH3BGRL3 expression between individuals (Figure 4.1). SH3BGRL3 is located on BTA 2. There was also a notable negative relationship (p=0.0168) between Apolipoprotein A-1 (APOA1) and RFI (regression coefficient=-1.98), with RFI value accounting for 53% of the variation in APOA1 expression. APOA1 is located on BTA 15. None of the other regression analyses were statistically significant.

4.4. Discussion

No single gene demonstrating differential expression between high and low RFI animals was expressed ubiquitously throughout all animals tested at a statistically significant level. Furthermore, neither upregulated or downregulated genes were differentially expressed at a statistically significant level in all of the animals in either the high or low RFI group. The lack of a clear RFI candidate gene for further study prompted further comparisons between the differentially expressed genes and RFI group.

The small number of animals involved in this study makes it difficult to draw definitive conclusions as to the contribution of specific genes to RFI in cattle. Due to the inherently large genetic variation between animals, the inconsistent upregulation/downregulation of common genes is not surprising, even considering individuals within the same RFI group. Future microarray studies should be expanded to include a much larger number of animals to validate the conclusions of this study. As

well, the oligonucleotide platform available for use in this experiment did not include IGF-1, which has been previously suggested as an indictor of RFI (Johnston et al. 2002; Wood et al. 2002). A larger selection of oligonucleotides to base further microarray studies upon is recommended.

As this is the first study using microarray technology to unearth potential candidate genes/markers for RFI, it is impossible to compare these results to other RFI or microarray studies. Nevertheless, it has been demonstrated that microarray technology can provide a great deal of useful information as to the genetic mechanisms behind certain biological processes. For example, Reverter et al. (2005) designed a comprehensive gene network defining the interactions between genes in bovine skeletal muscle. Other reviews of microarray progress in cattle (referring to recent Australian research and disease pathogenesis) can be found in Lehnert et al. (2006) and Wilson et al. (2005). With a thorough oligonuclotide platform and a considerable animal population to draw from, the same approach could easily be attempted in RFI research.

The function of SH3 domain-binding glutamic acid-rich protein like 3 (SH3BGRL3) has not yet been elucidated in cattle. Nonetheless, progress has been made as to its possible role in humans. While SHB3BGRL3 shares 25% identity and 63% conservation with glutaredoxin 1 (GRX1) of *Escherichia coli*, it lacks the conserved consensus sequence (CXXC) needed for reductase activity, indicating that it may be a modulator of glutaredoxin activity (Mazzocco et al. 2001). An interesting addendum to this point stems from knowledge of the glutaredoxin pathway. Glutaredoxin is responsible for converting glutathione to glutathione disulphide. Recycling of glutathione disulfide back to glutathione requires the reductive capacity of NADPH,

which is also required to maintain anti-oxidant protection in the mitochondria of cells (Outten and Culotta 2003). The findings of Kolath et al. (2006) revealed that mitochondrial function is more efficient in low RFI steers. It is possible that SH3BGRL3 has a regulatory effect upon the production or transfer of NADPH in the glutaredoxin pathway or in the process of anti-oxidant mitochondrial protection.

Apoptosis, proliferation, and senescence on the cellular level are all regulated to some extent by redox homeostasis, furthermore; GRX1 enhances the activation of the transcription factors nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1), which promote cell survival (Mazzocco et al. 2001). The sequence of SH3BGRL3 is identical to that of the C-terminal domain of tumour necrosis factor α (TNFα) inhibitory protein (TIP-B1), which protects cells from TNFα induced lysis (Berleth et al. 1999). In addition, it has recently been reported by Xu et al. (2005) that SH3BGRL3 may play a role in the regulation of the all-trans retinoic acid (ATRA)-induced pathway. ATRA is most commonly used in the treatment of acute promyelocytic leukemia (APL) to restore differentiation of affected cells (Zhang et al. 2000). Xu et al. (2005) demonstrated the upregulation of SH3BGRL3 in an APL cell line (NB4) treated with ATRA, indicating a regulatory role. ATRA is also implicated in the activation of the NF-κB pathway, suggesting that the expression of SH3BGRL3 stimulated by ATRA may be due to the activation of TNFα-induced NF-κB (Xu et al. 2005), which agrees with the similarity of SH3BGRL3 to GRX1.

The processes of differentiation, apoptosis, proliferation, and oxidative protection all require energy; therefore, it is hypothesized that the upregulation of SH3BGRL3, which may play a role in the protection of cells from degradation, could possibly reduce

the energy required for maintenance and turnover of said cells, perhaps allowing for more energy to be accessible for animal growth and production.

Apolipoprotein A-1 (APOA1) is the main protein component of the high density lipoproteins (HDLs) which play an important role in cholesterol transport from peripheral tissues to the liver and tissues responsible for steroidogenesis (e.g. adrenal cortex; Rigotti et al. 1997). For this transport to occur, cholesterol must first be converted into cholesterol esters by lecithin:cholesterol acyltransferase (LCAT), of which APOA1 is the major activator (O'hUigin et al. 1990). The system of reverse cholesterol transport by HDLs battles the buildup of cholesterol in the cardiovascular system; unsurprisingly, the bulk of research dealing with APOA1 and HDLs has focused upon the effects of these elements on atherosclerosis. This discussion, however, will concentrate on another aspect of APOA1, which is almost completely unrelated to cholesterol transport.

The outer membrane of Gram-negative bacteria releases endotoxin, a toxic lipopolysaccharide (Imai et al. 2003; Ma et al. 2004). During endotoxemia infection, symptoms in humans and animals are quite severe, often leading to shock and death (Imai et al. 2003). Ma et al. (2004) demonstrated that HDL limited the cytotoxicity of lipopolysaccaride, and that APOA1 performed the same function – concluding that APOA1 is the main component of HDL that protects cells from lipopolysaccaride toxicity.

Perhaps more interestingly, and in accordance with the hypothesized functions of SH3BGRL3, APOA1 also has a relationship with the TNFα pathway. During endotoxemia, the incidence of the proinflammatory cytokines, such as TNFα are greatly increased, which leads to cell degradation and death (Imai et al. 2003). Imai et al. 2003

discovered that the administration of APOA1 to rats experiencing endotoxin shock significantly decreased the release of TNFα by approximately 79% over 2 h, compared to a 34% reduction in TNFα in the control animals during the same time period. In addition, nine out of ten rats with endotoxemia which were treated with APOA1 survived for the next five days, while all ten control rats died within one day.

Immune response in an animal is a very energetically expensive process, and can require energy to be partitioned away from economic processes if an immune challenge is faced (Lochmiller and Deerenberg 2000). It is postulated that APOA1 may play a role in an improved general immune response, thus potentially allowing for more energy to be available for growth and production.

Table 4.1. Residual feed intake values (RFI)[1] of steers in the microarray study.

Steer ID	RFI Value (kg/d)
P1042	-2.49
P3021	-1.71
P3080	-1.57
P1023	-1.12
P1027	-1.05
P1084	-0.52
P3063	-0.28
P1081	-0.01
P1065	0.07
P1058	0.34
P3067	0.50
P1040	0.99
P1049	1.59

[1]Negative RFI values indicate a more feed efficient animal, positive RFI values indicate a less feed efficient animal.

Table 4.2. List of primers used in quantitative real-time polymerase chain reaction (QRT-PCR).

Gene ID	Forward Primer	Reverse Primer
Calponin 1	GGGTGAAGCCCCACGATAT	TGCGTGTGGTTGGTGTTCTC
Cofilin 2	CTCTTTTCCCACTGGGTCCTT	TGATAGGCTGCTTACATGAATGATATT
SH3 domain-binding glutamic acid-rich protein like 3	GGAAGCGCATCCAGTACCA	CCGCAGGGCGTTGTCTT
Pigment-epithelium derived factor	ACCCTCAGGCCGTTTTACG	GGGCGATCTTGCAGTTGAGA
Chemokine (C-C motif) ligand 20	CCCAGTATTCTTGTGGGCTTCA	GCATTGATGTCACAGGCTTCA
Protein kinase C, beta 1	TTCTTCAAGCAGCCCACCTT	CCCGAAGCCCCAGATGA
Filamin A	GGACCGCGAGAGCATCAA	CCGTCCACGATGGCCTTA
Fatty acid binding protein 4	GGAATGTGTCATGAATGGTGTCA	CCCTTGGCTTATGCTCTCTCA
Apolipoprotein-A1	TTTGGGAAAACAGCTCAACC	AGGTCCTTGTGCATCTCCTG

Table 4.3. List of probes used in quantitative real-time polymerase chain reaction (QRT-PCR).

Gene ID	Probe
Calponin 1	TTTGAGGCCAACGACCT
Cofilin 2	CAACACAATGAATGAAGG
SH3 domain-binding glutamic acid-rich protein like 3	CTAGTGGACATCTCC
Pigment-epithelium derived factor	TACGGCTTGGATTCTG
Chemokine (C-C motif) ligand 20	ACAGCAGCTGGCC
Protein kinase C, beta 1	AGCCACTGCACCGAC
Filamin A	CTCGTGTCCATCGACA
Fatty acid binding protein 4	TGCCACCAGAGTTT
Apolipoprotein-A1	GTTCTGGGACAACCTGGAAA

Table 4.4. Differential expression levels (fold change) of statistically significant[1] genes between low and high residual feed intake (RFI) groups[2].

Gene Name	Fold Change									
	Low RFI ID					High RFI ID				
	P1023	P1027	P1042	P1084	P3021	P3080	P1049	P1058	P3067	P1040
Chemokine (C-C motif) ligand 20				2.21		3.02	-2.71	-2.04	-2.25	
Protein kinase C, beta 1		-2.54	2.09					-3.43	-2.86	
Actin gamma 2, smooth muscle enteric		-4.02	2.37					-3.40	-3.26	
Calponin 1	-2.12	-3.36	1.59					-3.26	-2.90	
PDZ and LIM domain protein 3			2.18						-2.74	-4.34
Cofilin-2	-2.43	-3.83	1.98					-2.72	-3.21	
SH3 domain-binding glutamic acid-rich protein like 3	-1.77	-2.02	1.96				-2.70	-1.96		
Filamin A		-2.48	1.72					-2.36	-2.56	
B Myb			1.54					-2.21	-1.58	
F-box only protein 32		-1.55	1.59					-1.72		
Pituitary homeobox 2			1.67					-1.71		
Wiskott-Aldrich syndrome protein family member 3			1.55					-1.61		
Chemokine CC motif ligand 11		-1.82	1.56						-2.48	
Collagen, type III, alpha 1	-1.55	-1.78	2.39	1.64					-2.28	-1.61
Pigment epithelium derived factor	-1.52	-1.66	1.54	1.72					-2.24	-2.34
Pyruvate dehydrogenase kinase, isoenzyme 4				1.58					-2.16	
FK506 binding protein 5			1.64	2.17	-1.63				-2.05	

[1] $p \leq 0.05$

[2] Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d

Table 4.4 (con't). Differential expression levels (fold change) of statistically significant[1] genes between low and high residual feed intake (RFI) groups[2].

Gene Name	Fold Change									
	Low RFI ID						High RFI ID			
	P1023	P1027	P1042	P1084	P3021	P3080	P1049	P1058	P3067	P1040
Caveolin 1	-1.51	-1.66	1.71						-1.77	
Osteonectin			1.65						-1.56	
Fatty acid binding protein 4					-1.40	1.67				-1.72
LOC387758 protein			1.89						-1.76	
Apolipoprotein A-I					2.31	2.10		-2.12	-2.33	

[1] $p \leq 0.05$

[2] Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d

Table 4.5. Quantitative real-time polymerase chain reaction (QRT-PCR) results.

Gene Name	Animal	RFI Group[1]	ΔCT[2]
Chemokine (C-C motif) ligand 20	P1023	Low	-4.27
	P1027	Low	-3.53
	P1042	Low	0.48
	P1084	Low	0.73
	P3021	Low	-4.63
	P3080	Low	1.08
	P1049	High	-6.86
	P1058	High	-2.69
	P3067	High	-7.42
	P1040	High	-1.94
Calponin 1	P1023	Low	-4.96
	P1027	Low	-1.90
	P1042	Low	3.58
	P1084	Low	1.20
	P3021	Low	1.69
	P3080	Low	2.40
	P1049	High	1.06
	P1058	High	-2.44
	P3067	High	-3.78
	P1040	High	-0.56
Cofilin 2	P1023	Low	-9.05
	P1027	Low	-5.93
	P1042	Low	-0.58
	P1084	Low	-3.88
	P3021	Low	-3.66
	P3080	Low	-2.83
	P1049	High	-3.54

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]ΔCT = # amplification cycles of internal standard - # amplification cycles of target

Table 4.5 (con't). Quantitative real-time polymerase chain reaction (QRT-PCR) results.

Gene Name	Animal	RFI Group[1]	ΔCT[2]
Cofilin 2	P1040	High	-5.39
	P1058	High	-6.19
	P3067	High	-8.11
SH3 domain-binding glutamic acid-rich protein like 3[3]	P1023	Low	2.51
	P1027	Low	4.40
	P1042	Low	3.16
	P1084	Low	-0.11
	P3021	Low	2.55
	P3080	Low	2.12
	P1049	High	-0.47
	P3067	High	0.39
	P1040	High	-0.70
Pigment epithelium derived factor	P1023	Low	-5.82
	P1027	Low	-0.96
	P1042	Low	-0.54
	P1084	Low	-0.80
	P3021	Low	-2.52
	P3080	Low	-3.85
	P1049	High	-0.64
	P1058	High	-4.61
	P3067	High	-9.63
	P1040	High	-5.45
Protein kinase C, beta 1	P1023	Low	-7.30
	P1027	Low	-7.60
	P1042	Low	2.11
	P1084	Low	-1.37
	P3021	Low	-2.72

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]ΔCT = # amplification cycles of internal standard - # amplification cycles of target
[3]Sample from P1058 did not amplify properly, was excluded from results

Table 4.5 (con't). Quantitative real-time polymerase chain reaction (QRT-PCR) results.

Gene Name	Animal	RFI Group[1]	ΔCT[2]
Protein kinase C, beta 1	P3080	Low	-2.73
	P1049	High	-1.52
	P1058	High	-4.89
	P3067	High	-8.72
	P1040	High	-2.87
Filamin A	P1023	Low	-5.20
	P1027	Low	-5.01
	P1042	Low	1.50
	P1084	Low	-0.53
	P3021	Low	-0.61
	P3080	Low	-1.22
	P1049	High	-0.57
	P1058	High	-6.31
	P3067	High	-6.70
	P1040	High	-1.35
Fatty acid binding protein 4	P1023	Low	0.56
	P1027	Low	1.40
	P1042	Low	-0.60
	P1084	Low	-0.01
	P3021	Low	-0.98
	P3080	Low	0.90
	P1049	High	-0.37
	P1058	High	-1.47
	P3067	High	-3.51
	P1040	High	-1.56
Apolipoprotein A-1	P1023	Low	-12.43
	P1027	Low	-6.66

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]ΔCT = # amplification cycles of internal standard - # amplification cycles of target

Table 4.5 (con't). Quantitative real-time polymerase chain reaction (QRT-PCR) results.

Gene Name	Animal	RFI Group[1]	ΔCT[2]
Apolipoprotein A-1	P1042	Low	-3.61
	P1084	Low	-9.72
	P3021	Low	-4.80
	P3080	Low	-3.64
	P1049	High	-10.80
	P1058	High	-10.58
	P3067	High	-13.12
	P1040	High	-9.45

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]ΔCT = # amplification cycles of internal standard - # amplification cycles of target

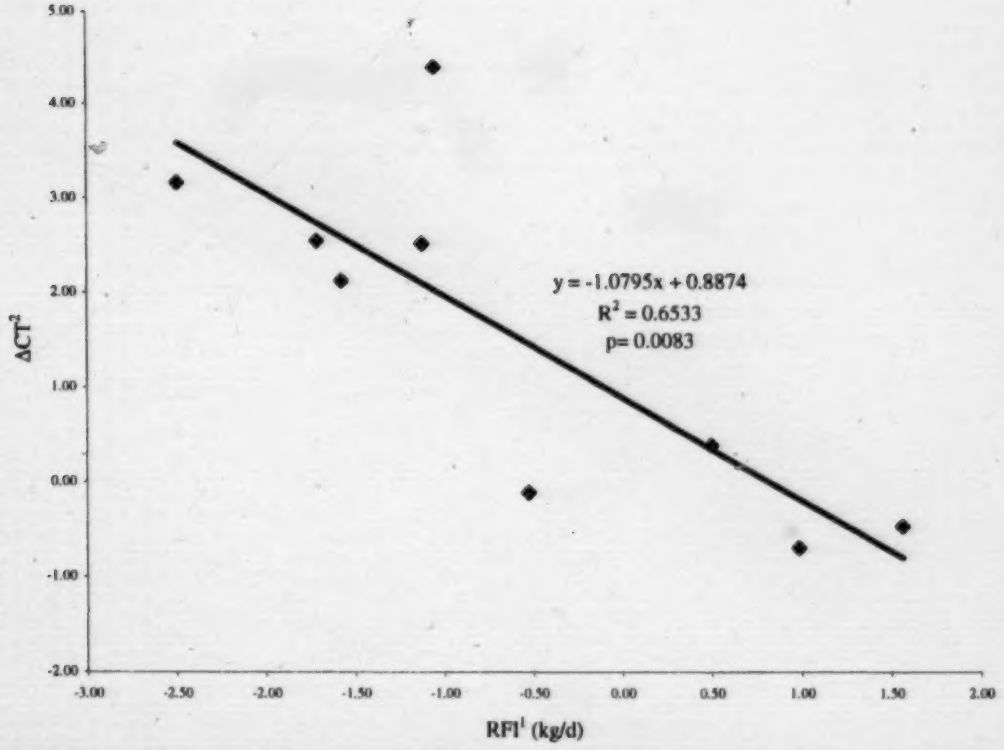

Figure 4.1. Regression analysis of SH3 domain-binding glutamic acid-rich protein like 3 (SH3BGRL3) quantitative real-time polymerase chain reaction (QRT-PCR) results against residual feed intake (RFI).

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]ΔCT = # amplification cycles of internal standard - # amplification cycles of target

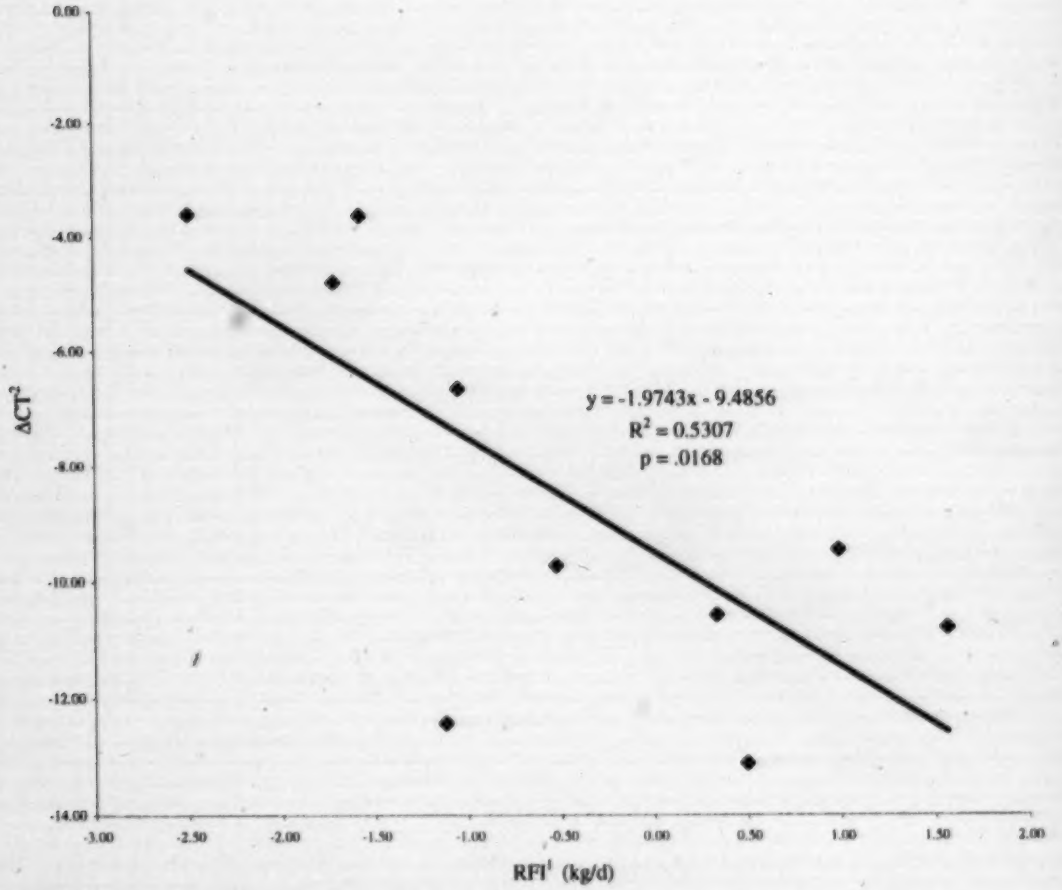

Figure 4.2. Regression analysis of Apolipoprotein A-1 (APOA1) quantitative real-time polymerase chain reaction (QRT-PCR) results against residual feed intake (RFI).

[1]Low RFI values = -1.41 ± 0.678 kg/d, high RFI values = 1.02 ± 0.535 kg/d
[2]ΔCT = # amplification cycles of internal standard - # amplification cycles of target

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Chapter 5

General discussion and conclusions

5.1. General discussion and conclusions

Improving feed efficiency in beef cattle has the potential to have a sizeable impact upon production costs, as feed accounts for 60-70% of the total input costs in beef production systems (Lindsay 2006). Recently, many research efforts have been aimed at discovering the physiological and genetic mechanisms underlying feed efficiency, in the hopes of revealing a cost effective method by which to select the most efficient animals (Archer et al. 1999, Arthur et al. 2004, Crews 2005). Residual feed intake (RFI) is a measure of feed efficiency that is unrelated to growth rate or mature size (Archer et al. 1999), signifying that direct selection for feed efficiency based upon this indicator measure would not increase the maintenance requirements of the animal, nor sacrifice growth rate (Herd and Bishop 2000). The objective of this study was to examine metabolic and genetic factors contributing to the variation between individuals in RFI, and perhaps provide a preliminary starting point for the development of selection criteria for RFI and feed efficiency.

Chapter 3 evaluated the relationship of RFI with methane production, heat production, and skeletal muscle protein breakdown. It was hypothesized that low RFI steers would have a lower methane and heat production, as well as a lower rate of protein degradation. However, the results from this study were inconsistent with published literature, as there was no statistically significant relationship between RFI and methane production, heat production or 3-methylhistidine concentration.

Hotovy et al. (1991), Herd et al. (2002), Basarab et al. (2003), Okine et al. (2003), Hegarty et al. (2004), and Nkrumah et al. (2006) report a significant effect of RFI group upon methane production and heat production, with low RFI animals producing a lower amount of each. If low RFI animals retain more energy because less is lost as methane and/or heat, it stands to reason that they would have improved feed efficiency. The correlation between dry matter intake and heat production reported in this study is supported by the research of Nkrumah et al. (2006). This author speculates that the small sample size in this study is responsible for the unexpected results.

Urinary 3-methylhistidine (3-MeH) is an accepted method of determining protein degradation in skeletal muscle (Nishizawa et al. 1979; Harris and Milne 1981; McCarthy et al. 1983), however; in this study, the day-to-day 3-MeH production was much too variable to draw any conclusive results. It has been suggested that 67% of all maintenance requirements in cattle are due to ion transport and protein metabolism (Herd et al. 2004). In addition, Oddy et al. (1998) determined that more feed efficient steers have a lower rate of protein degradation. Based on reports in the literature, it was expected that low RFI steers would have demonstrated a lower rate of protein degradation in skeletal muscle, thus expending less energy for protein metabolism, and resulting in improved feed efficiency. Although there was no statistically significant difference in urinary 3-MeH output between low and high RFI steers, which supports the results of Richardson et al. (2004), this is most likely due to large fluctuations in total urine output by the steers over the sampling period brought on by stress. It is possible that a larger sample population could discern these differences in 3-MeH production.

Chapter 4 examined 8700 genes that could have an impact on RFI. With the rapid advances in quantitative trait loci (QTL) studies (Casas et al. 2000; Moore et al. 2003; Kneeland et al. 2004; Li et al. 2004; Hoque et al. 2005; Nkrumah 2006) in cattle, more interest has been aroused in high throughput genetic analysis, such as microarrays. The greatest advantage of microarray technology lies in its ability to analyze thousands of genetic elements at once time, even without any prior knowledge of the genetic mechanisms regulating the subject of interest (Lehnert et al. 2006). Currently, the determination of feed efficiency through the measurement of RFI is costly and time consuming. A genetic marker or selection criteria for RFI would greatly reduce both the cost and time commitment involved with differentiating feed efficient and feed inefficient animals. This was the first study to examine differential expression of genes between low and high RFI steers with the assistance of microarray technology in beef cattle.

The results from the microarray identified 22 candidate genes that were differentially expressed between low and high RFI animals. However, no one gene was expressed at a statistically significant level in all of the 10 animals tested. In addition, while all 22 genes demonstrated a significant downregulation in high RFI steers, some of the same genes in the low RFI steers were either upregulated (as would be expected) or downregulated in different low RFI individuals. There was no clear consensus data indicating a potential candidate gene for RFI. To clarify these results, nine genes were chosen to validate the microarray results using quantitative real time polymerase chain reaction (QRT-PCR). These genes were chosen based upon the microarray results, namely, how many animals the gene was expressed at a significant level in, and if the upregulation/downregulation phenomenon was present in the low RFI group.

Results of QRT-PCR validation generally confirmed the microarray results, although, as the QRT-PCR was performed on all 10 animals and not just the ones that demonstrated significant gene expression in the microarray study, it became clear that upregulation or downregulation of the same gene in a particular RFI group was inconsistent. Regression of the QRT-PCR results against RFI value revealed two genes that had a significant negative relationship with RFI: SH3 domain-binding glutamic acid-rich protein like 3, and apolipoprotein A-1 (APOA1).

Although the function of SH3BGRL3 is presently unknown in cattle, it has been implicated in three main pathways in humans. Firstly, SH3BGRL3 shares a great deal of similarity with glutaredoxin 1 of *Escherichia coli,* though it lacks the conserved consensus sequence necessary for glutaredoxin reductase activity. This indicates that SH3BGRL3 may be a modulator of the glutaredoxin pathway (Mazzocco et al. 2001). Secondly, the sequence of SH3BGRL3 is identical to that of the C-terminal domain of tumour necrosis factor α (TNFα) inhibitory protein (TIP-B1), which protects cells from TNFα induced lysis (Berleth et al. 1999). Thirdly, SH3BGRL3 is implicated in the regulation of the all-trans retinoic acid induced pathway, which restores differentiation in cells affected with acute promyelocytic leukemia (Xu et al. 2005).

The cellular processes of differentiation, apoptosis, and proliferation all require energy to maintain proper function. If cells are protected from premature degradation by the upregulation of SH3BGRL3, it is postulated that this may reduce the energy required for routine cell maintenance and turnover, possibly resulting in more available energy for animal growth and production.

Reverse cholesterol transport involves the transformation of cholesterol to cholesterol esters by lecithin:cholesterol acyltransferase (LCAT), and transport of these esters to the liver or steroidogenic tissues by high density lipoproteins (HDLs; Rigotti et al. 1997). A main component of HDL and major activator of LCAT is APOA1 (O'hUigin et al. 1990; Rigotti et al. 1997). In addition, APOA1 is the main component of HDL that protects cells from lipopolysaccaride toxicity and endotoxemia (Imai et al. 2003; Ma et al. 2004). APOA1 is also involved in the TNFα pathway, much like SH3BGRL3. Imai et al. (1993) demonstrated that rats with endotoxemia were much less likely to die if treated with APOA1, due to the reduction in circulating TNFα instigated by APOA1.

As the immune response is an energetically expensive process (Lochmiller and Deerenberg 2000), if an animal is better suited to dealing with immune challenge, less energy would be expended trying to fight off or recover from an infection. It is hypothesized that APOA1 expression plays a role in immune response that may be beneficial in terms of feed efficiency.

The results of this study indicate that there is genetic variation between animals in terms of RFI and feed efficiency. However, this study was unable to confirm previously recorded differences in metabolic measurements for steers varying in RFI value, such as the decreased methane and heat production of low RFI steers reported by Nkrumah et al. (2006). This project also provides two preliminary candidates for the genetic selection of feed efficiency. As this is the first study to apply microarray technology in the analysis of the genetic components influencing RFI, more research must be completed to validate or refute the conclusions drawn herein. With a larger sample population and a thorough

oligonucleotide microarray platform, experiments of this type have the potential to greatly advance feed efficiency research. It is would also be beneficial to further explore the potential relationship between the glutaredoxin pathway and mitochondrial function alluded to in Chapter 4 of this thesis.

Feed efficiency is a highly complex trait, heavily influenced by environmental conditions; therefore, it is unlikely that selection against one or two genes will be sufficient to effect a noticeable change upon feed efficiency. There is no doubt that genomic technology has enormous potential to further feed efficiency research, and these avenues should be explored fully; keeping in mind the benefits to producers, as well as the potential drawbacks of overselection.

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Appendices

Appendix 1

Total RNA Isolation from Animal Tissue using TRIzol Reagent

1. Cool mortar and pestle in -20°C overnight.
2. Clean the homogenizer probe (Ultra Turrax T18 basic, IKA Works, Inc., North Carolina, USA) with RnaseZap, 0.1N NaOH, sterile milliQ water, and 100% (v/v) ethanol.
3. Weigh 14 mL Falcon tube(s). Cool in liquid nitrogen.
4. Grind frozen tissue using the pre-chilled mortar and pestle on a bed of dry ice to a fine powder. Add liquid nitrogen to the sample to keep chilled.
5. Transfer approximately 500 mg ground tissue into the pre-chilled 15 mL Falcon tubes. Keep on dry ice.
6. Add 5 mL TRIzol to each tube, vortex briefly to suspend tissue.
7. Homogenize samples (Speed 6, 24 000 RPM) for 5-10 s, four times. Keep samples on ice.
8. Incubate samples at room temperature for 20 min.
9. Centrifuge at 12,000 x g, 4°C for 10 min. Pour supernatant into new 14 mL tube. Add 1 mL chloroform. Vortex briefly, incubate at room temperature for 2-3 min.
10. Centrifuge at 12,000 x g, 4°C for 15 min. Transfer clear supernatant into new 14 mL tube.
11. Add 1.25 mL isopropyl alcohol, 1.25 mL high salt solution (1.2M NaCl, 0.8M $C_6H_5Na_3O_7$). Vortex briefly and incubate at room temperature for 15 min.
12. Centrifuge at 12,000 x g, 4°C for 10 min. Pour off supernatant. Invert tube to drain.

13. Wash pellet with 5 mL 75% (v/v) ethanol. Centrifuge at 8300 RPM, 4°C for 3 min. Pour off supernatant. Solubilize pellet in 300 µL DEPC treated water. Pool RNA solution into one 14 mL tube.
14. Add 0.1 X volume 3M sodium acetate and 2.5 X volume 100% (v/v) ethanol. Incubate at -20°C overnight.
15. Centrifuge at 12,000 x g, 4°C for 20 min. Remove supernatant. Dry pellet at room temperature for 5-10 min.
16. Repeat ethanol precipitation one more time.
17. Solubilize pellet in 300 µL DEPC treated water and store at -80°C.

Appendix Two

Sample Preparation for Microarray

PART I. Reverse transcription to synthesize first strand cDNA.

1. Place 10 µL RNA (1µg) into a nonstick, sterile, RNase-free 0.5 mL tube. Add 1 µL T7 Oligo(dT) Primer and 1 µL nuclease-free water. Vortex briefly, and centrifuge to collect mixture at bottom of tube.
2. Incubate for 10 min at 70°C in a thermal cycler. Centrifuge for ~5 s and place on ice.
3. Add 8 µL Reverse Transcription Master Mix (2 µL 10X first strand buffer, 4 µL dNTP mix, 1 µL RNase inhibitor, 1 µL Array script). Mix thoroughly by pipetting 2-3 times, flicking the tube 3-4 times, and centrifuge to collect reaction at bottom of tube. Place samples in hybridization oven for 2 h at 42°C.
4. After incubation, centrifuge briefly to collect the reaction at the bottom of the tube. Place the tubes on ice and proceed to Part II.

PART II. Second strand cDNA synthesis.

1. Add 80 µL of Second Strand Master Mix to each sample (63 µL nuclease-free water, 10 µL 10X second strand buffer, 4 µL dNTP mix, 2 µL DNA polymerase, 1 µL RNase H. Mix thoroughly by pipetting 2-3 times, flicking the tube 3-4 times, and centrifuge to collect reaction at bottom of tube. Incubate in thermal cycler at 16°C for 2 hr.
2. Place reactions on ice or freeze at -20°C.

PART III. cDNA purification.

1. Preheat nuclease-free water to 50°C for at least 10 min.

2. Add 250 µL cDNA binding buffer to each sample. Mix thoroughly by pipetting 2-3 times, flicking the tube 3-4 times, and centrifuge to collect reaction at bottom of tube.
3. Place cDNA filter cartridge into wash tube. Pipet cDNA sample from step 2 onto the center of filter cartridge. Centrifuge for 1 min at 10 000 x g. Discard flow through and replace filter cartridge in wash tube.
4. Wash filter cartridge with 500 µL wash buffer. Centrifuge for 1 min at 10 000 x g. Discard flow through and replace filter cartridge in wash tube. Centrifuge again for 1 min at 10 000 x g to remove any last wash buffer. Transfer filter cartridge to cDNA elution tube.
5. Pipet 9 µL of pre-heated (step 1) nuclease-free water to center of filter cartridge. Leave at room temperature for 2 min, then centrifuge for 1.5 min at 10 000 x g. Repeat and discard filter cartridge.

PART IV. In vitro transcription to synthesize aRNA.

1. Add 26 µL of IVT Master Mix to each sample (3 µL 50mM aaUTP, 12 µL 25mM ATP, CTP, GTP mix, 3 µL 50 mM UTP solution, 4 µL T7 10X reaction buffer, 4 µL T7 enzyme mix). Mix thoroughly by pipetting 2-3 times, flicking the tube 3-4 times, and centrifuge to collect reaction at bottom of tube.
2. Incubate in hybridization oven for 16 h at 37°C.
3. Add 60 µL nuclease-free water to each sample and mix by vortexing gently. Volume should be 100 µL.

PART V. aRNA purification.

1. Preheat nuclease-free water to 50°C for at least 10 min.
2. Add 350 µL aRNA binding buffer to each sample.
3. Add 250 µL 100% (v/v) ethanol and pipet 3 times to mix.
4. Place aRNA filter cartridge into collection tube. Pipet mixture from step 3 onto center of filter cartridge. Centrifuge for 1 min at 10 000 x g. Discard flow through and replace filter cartridge in collection tube.
5. Wash with 650 µL wash buffer. Centrifuge for 1 min at 10 000 x g. Discard flow through and replace filter cartridge in wash tube. Centrifuge again for 1 min at 10 000 x g to remove any last wash buffer. Transfer filter cartridge to new aRNA collection tube.
6. Pipet 100 µL of pre-heated (step 1) nuclease-free water to center of filter cartridge. Leave at room temperature for 2 min, then centrifuge for 1.5 min at 10 000 x g. Repeat and discard filter cartridge.

<u>PART VI. aRNA:Dye Coupling Reaction (MAKE SURE ROOM IS DARK AS POSSIBLE).</u>

1. Resuspend Cy3 and Cy5 dye in 12 µL of DMSO and vortex.
2. Determine aRNA concentration of samples and vacuum dry 10 µg. Do not over dry.
3. Resuspend dried aRNA in 9 µL coupling buffer and vortex gently.
4. Add 11 µL prepared dye mix to aRNA and mix well by vortexing gently.
5. Incubate for 1 hour at room temperature.
6. Add 4.5 µL Hydoxylamine and mix by vortexing gently.
7. Incubate 15 min at room temperature.

8. Add 5.5 µL nuclease-free water. Volume should be 30 µL.

PART VII. Dye Labeled aRNA Purification.

1. Preheat nuclease-free water to 50°C for at least 10 min.
2. Add 105 µL aRNA binding buffer to each sample.
3. Add 75 µL 100% (v/v) ethanol and pipet 3 times to mix.
4. Place labeled aRNA filter cartridge into labeled collection tube. Pipet mixture from step 3 onto center of filter cartridge. Centrifuge for 1 min at 10 000 x g. Discard flow through and replace filter cartridge in labeled collection tube.
5. Wash with 500 µL wash buffer. Centrifuge for 1 min at 10 000 x g. Discard flow through and replace filter cartridge in wash tube. Centrifuge again for 1 min at 10 000 x g to remove any last wash buffer. Transfer filter cartridge to new labeled aRNA elution tube.
6. Pipet 10 µL of pre-heated (step 1) nuclease-free water to center of filter cartridge. Leave at room temperature for 2 min, then centrifuge for 1.5 min at 10 000 x g. Repeat twice and discard filter cartridge.
7. Determine concentration of aRNA and vacuum dry 5 µg.
8. Pre-Hybridize microarray slides in pre-hybridization buffer for 45 min at 42°C (2.5 g BSA, 186.5 mL RNase free water, 62.5 mL 20X SSC, 1.25 mL 10% (v/v) SDS - Mix buffer, filter sterilize it, and warm to 42°C).
9. Place slides into new holder. Wash in purified water for 5 min at 50 RPM. Repeat twice with fresh water each time. Dip slides into isopropanol 2-3 times, then spin dry. Add cover slip and preheat to 42°C.

PART VIII. Preparing labeled aRNA for hybridization.

1. From step 7 of Part VII, make sure that labeled aRNA samples do not dry completely. Leave ~9 µL – if dried to a lower volume, add nuclease free water to increase to 9 µL.
2. Add 1 µL fragmentation buffer.
3. Incubate for 15 min at 70°C.
4. Add 1 µL stop solution and place on ice until use.
5. Add 27 µL 30% (v/v) formamide buffer to each of the fragmented labeled aRNA samples (22.5 µL deionized formamide, 18.75 µL 20X SSC, 0.75 µL 10% (v/v) SDS), 6 µL 10 mg/mL yeast tRNA, 6 µL 10 mg/mL salmon sperm DNA). Pulse spin to remove bubbles.
6. Slowly add mixture made in step 5 to one side of the coverslip on the microarray slides, ensure no formation of bubbles.
7. Place slides in hybridization chamber and put chamber in hybridization oven for 16-20 h at 42°C.

STEP IX. Washing microarray slides after hybridization.

1. Wash slides twice with low stringency wash buffer (2X SSC, 0.5% (v/v) SDS) for 5 min at 50 RPM, using fresh buffer each time.
2. Wash slides twice with high stringency wash buffer (0.5X SSC, 0.2% (v/v) SDS) for 5 min at 50 RPM, using fresh buffer each time.
3. Transfer slides to new holder and wash once with final wash (0.05X SSC) for 5-7 min at 50 RPM. Spin slides dry and scan as soon as possible.

Appendix Three

Quantitative Real-Time Polymerase Chain Reaction

PART I. DNase treatment.

1. Add 1 µL of DNase.
2. Add 1 µL 10 X buffer.
3. Add 8 µL of 100 ng/µL RNA.
4. Incubate at room temperature for 15 min.
5. Add 1 µL of 25 mM EDTA.
6. Incubate at 65°C for 10 min, then snap chill on ice.

PART II. Reverse transcription to synthesize cDNA.

1. Put 10 µL of DNase treated RNA in a PCR tube.
2. Add 0.5 µL of oligo DT (1 ug/µL).
3. Add 1 µL of dNTPs (20 mM).
4. Add 1 µL of RNase free water.
5. Heat to 65°C, followed by snap chill on ice.
6. Add 4 µL of 5 X first strand buffer.
7. Add 0.5 µL of RNase OUT.
8. Add 2 µL of DTT (0.1 mM).
9. Incubate at 42°C for 2 min.
10. Add 1 µL of Superscript II.
11. Incubate at 42°C for 50 min.
12. Incubate at 70°C for 15 min.

PART III. Quantitative real-time PCR.

Add the following to each well in 96 well PCR plate (in triplicate for each specific set of primers and probes used):

1. 12.5 µL of PCR master mix.
2. 0.225 µL specific forward primer (100 µM).
3. 0.225 µL specific reverse primer (100 µM).
4. 0.05 µL fluorescent probe (100 µM).
5. 7 µL DNase free water.
6. 3 µL cDNA from Part II.
7. If using positive and negative controls, leave some wells free of cDNA. For positive controls add 2.5 µL 10 X Exo IPC Mix and 0.5 µL of 50 X Exo IPC DNA instead of cDNA to the above mixture. For negative controls fill wells with all reagents above, except cDNA.
8. Seal PCR plate with optical adhesive covers, centrifuge for ~1 min to remove bubbles and place in ABI Prism 7700 Sequence Detection System. Close lid.
9. Thermal cycler conditions as follows:

   Hold 2 min at 50°C.

   Hold 10 min at 95°C.

   Cycle (each of 40) 15 s at 95°C.

   Cycle (each of 40) 1 min at 60°C.